NOTES ON THE BURSTING STRENGTH OF THE ALIMENTARY TRACT OF THE CAT

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FOUR FIGURES

INTRODUCTION

Comparatively little attention has been paid by anatomists to the questions of the strength of tissues, and to the mechanical factors concerned in such problems, except in the correlation of the architecture of bones with their tensile strength, and in the mechanism of joints, as developed by Fick in Bardeleben's 'Handbuch der Anatomie.' Anatomists have seemingly neglected this aspect of the physiological anatomy of the alimentary canal, and surgeons alone seem to have been interested in it to any great extent. The experiments performed by these investigators have been, however, rather meagre. Ruptures of the hollow viscera by filling them to various degrees of tension with air or water, and then striking them with a hammer or board have been produced. By this method the blow causes the organ to flatten out both on the side struck and on the side opposite, and so modifies, as will be shown later, the effect of the internal pressure upon the walls. For this procedure increases the tendency to produce the tear along the edge which is made more round by the blow.

These experiments are, therefore, not identical with those in which the rupture is produced by merely increasing the pressure within the organ by a gas or a fluid. Such pure bursting ruptures have been produced experimentally by only a few observers upon the stomach, intestines and oesophagus of man, dog and pig. Key-Aberg,[1] using 30 human stomachs obtained

[1] A. Key-Aberg. Zur Lehre der spontanen Magenruptur. Nord. Medic. Arkiv., Bd. 22, 1890.

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from fresh cadavers, found that the stomach would rupture when the internal pressure was equal to a column of water $1\frac{1}{2}$ to 2 meters high (from 1/7 to 1/5 of an atmosphere); the tear produced was quite constantly longitudinal, on or near the lesser curvature, beginning first in the mucosa near the cardia. Hertle[2] gives his results on six loops of dog intestine and three loops of pig intestine. Those of the dog ruptured at a pressure of 300 to 560 mm. Hg., the tear in each case being longitudinal through all the layers, and most commonly situated on the non-mesenteric border. Those of the pig ruptured at 260 to 300 mm. Hg., were also longitudinal, but the tear was near the mesenteric border. Brosch[3] found the result of over-distension of ten human oesophagi to be a longitudinal tear, beginning in the submucosa. He gives no figures as to the strength of the oesophagus.

METHOD OF PROCEDURE AND MATERIAL USED

In these observations the fresh material from seventeen adult cats, killed by ether or chloroform, was used immediately after death. In all cases peristalsis of the intestines could still be obtained. Loops of small intestine, about 7 cm. long, were carefully cut from the mesentery close to the intestinal attachment. In addition to these loops, consisting of all the coats of the intestine, preparations were made of loops deprived of the muscle and peritoneal layers, and so consisting of submucosa and mucosa alone. On account of the meagre fibrous connections between the muscles and submucosa (evident in any histological preparation) the muscle layers together with the peritoneum may be easily stripped off, leaving the submucosa and mucosa intact.

In the routine observation the oesophagus was dissected out of the thorax and removed together with the stomach. The stomach was isolated by ligating the oesophagus at the cardia. In one experiment the oesophagus and stomach were left in situ,

[2] Josef Hertle. Über stumpfe Verletzungen des Darmes und des Mesenteriums. Beiträge zur klinischen Chirurgie, Bd. 53, 1907.

[3] A. Brosch. Die spontane Ruptur der Speiseröhre auf Grund neuer Untersuchungen. Virchow's Archiv, Bd. 162, 1900.

the thorax not being opened, and the pylorus tied off. The greater and lesser omenta in all cases were carefully removed from the stomach. The caecum was cut from the colon about 6 cm. from the tip of the caecum, and the fat and connective tissue bands running from the caecum to the ileum were carefully dissected off.

The pressure to produce the rupture was obtained from the water tap, which afforded a pressure as high as 75 lbs. per square inch. From the faucet the water was led through iron pipes (found necessary to prevent bursting) to pressure gauges and to the glass cannula on which the intestines and the other hollow viscera were tied. The cannula and gauges were all placed on the same horizontal tubing, thus obviating almost completely any hydrostatic errors. It was found that ordinary steam gauges, registering respectively 30 and 150 lbs. to the square inch, were quite satisfactory.[4] The system was completely closed except for the opening of the cannula. On this, the trial loops were placed with the opposite end strongly closed by a hemostat.

In the case of the oesophagus the water was allowed to flow in from the upper end, the cardiac end being tied. In the observations on the stomach the water entered the pylorus after closure of the cardiac end. When the caecum was used the water entered it through the cut end where it had joined the colon; the junction of ileum with the caecum was tied off.

Preparations of the submucosa of the intestine were made for the purpose of studying the direction of its connective tissue fibers. Loops of intestine were filled to various degrees of distension with Carnoy's fixing fluid. The muscle layers and the mucosa were then dissected off, leaving the submucosa. Pieces of this were placed in very dilute phosphomolybdic acid for several hours, washed in distilled water, and then placed over night in dilute aniline blue solution. The specimens were then

[4] The pressures necessary for rupture were obtained in these experiments in terms of pounds per square inch. These figures have been converted to the corresponding pressures in terms of millimeters of mercury, considering atmospheric pressure equal to 760 mm. Hg., or 14.7 lbs. per square inch. They have been placed in parentheses after the first figures obtained.

dehydrated in absolute alcohol, cleared in benzol and mounted in damar. Histological sections of small intestine both longitudinal and transverse, stained for connective tissue by Mallory's method, were also prepared.

RESULTS OBTAINED

Using the technic outlined above it was found that the pressure necessary to rupture the oesophagus varied from the lowest 13 lbs. (672 mm. Hg.) in one instance, to the highest 24 lbs. (1240 mm. Hg.) in another case. The average of all fourteen cases was 17.75 lbs. (911 mm. Hg.). The tear was constantly longitudinal, extending through all the coats, with clean-cut edges, beginning on the average 5.0 cm. from the cardia and extending upward for 2.0 cm. The oesophagus apparently lengthened but little, whereas it dilated markedly.

The stomach dilated at first very markedly before the pressure within it rose appreciably. Its point of rupture was then, however, soon reached, varying only slightly in the fifteen cases from the average found—3.73 lbs. (193 mm. Hg.). Nine times the tear was on or near the lesser curvature, and six times, on or near the greater curvature. (Once its position was not noted with reference to the curvatures of the stomach.) In all instances the tear had clean-cut edges, extended through all the coats of the stomach, and ran parallel with either greater or lesser curvatures. Nine times out of fifteen, the tear was nearer to the cardiac end than to the pyloric end. In one additional case, where the stomach and oesophagus had been left in situ, and the thorax not opened (in a living animal), the cannula was inserted into the oesophagus in the neck, and the pylorus tied off. Rupture of the stomach occurred at 2.5 lbs. pressure (129 mm. Hg.) along the greater curvature, 1.0 cm. in front of the omental attachment, and parallel with this border. The tear was 3.0 cm. in length. The oesophagus remained undamaged.

In 32 trials, pieces of jejunum were used having all their coats intact. The lowest pressure to produce rupture was 17.5 lbs. (905 mm.) in one cat, the average of all cases being 28.2 lbs.

(1458 mm.). In each individual cat, however, the figures were more constant; the greatest difference was in one cat where two trials of intact jejunum gave 32 lbs. (1654 mm.) at one time, and 37 lbs. (1913 mm.) at another. Such duplicate tests were made in twelve other cats; most commonly there was a variation of only 1 or 2 lbs. (52 mm.–104 mm.) in any one individual.

The total average of 18 trials of portions of the ileum with all coats intact was 24.8 lbs. (1282 mm.). The results ranged from 17.5 lbs. (905 mm.), the lowest figure, to 37.5 lbs. (1939 mm.) which was the highest figure obtained. Here also, where duplicate tests were made in one and the same cat, the greatest difference was only 6 lbs. (310 mm.) in a cat where once the ileum ruptured at 21.5 lbs. (1112 mm.), and the second time at 27.5 lbs. (1422 mm.). In three duplicate tests the figures either agreed or varied only a few pounds.

The caecum showed considerable distensibility, and its point of rupture varied from 8.75 lbs. (452 mm.), which was the lowest figure, to 25 lbs. (1292 mm.), the highest figure obtained. The average of twelve cases was 16.9 lbs. (874 mm.). In six cases the tear was along the edge opposite the ileo-caecal valve, and in six cases along the edge on which the ileo-caecal valve is situated.

The following table (No. 1) gives the results of rupturing the intact oesophagus, stomach, small intestine and caecum of the cat, giving also the average length of the tear, and in the case of the intestines, the distance the tear was situated from the mesenteric border.

In regard to the nature of the tear it was seen that by slowly increasing the pressure in those intestines which ruptured at a relatively low point, for instance at 18 lbs. (931 mm.), the muscle coats, serosa, submucosa and mucosa, ruptured practically simultaneously, so that it was difficult to say which tore first. In those cases, however, where the rupture of the submucosa took place at a greater pressure, in one case for instance at 43 lbs. (2223 mm.), the muscle coats had already ruptured when the pressure had reached 33 lbs. (1706 mm.). The tears in the muscle layers were usually somewhat longer than the tears in

TABLE 1

Alimentary tract, intact

	NUMBER OF TRIALS	LOWEST PRESSURE PRODUCING RUPTURE	HIGHEST PRESSURE PRODUCING RUPTURE	AVERAGE PRESSURE NECESSARY TO PRODUCE RUPTURE	LOWEST AND HIGHEST PRESSURES IN AN INDIVIDUAL SHOWING GREATEST DIFFERENCE IN DUPLICATE TESTS	NUMBER OF DUPLICATE TESTS MADE	AVERAGE LENGTH OF TEAR	AVERAGE DISTANCE OF TEAR FROM MESENTERIC BORDER
Oesophagus...	14.	13 lbs. (672 mm.)	24 lbs. (1240 mm.)	17.75 lbs. (911 mm.)			2.0 cm.	
Stomach.....	15.	2.75 lbs. (135 mm.)	5 lbs. (259 mm.)	3.7 lbs. (193 mm.)			2.6 cm.	
Jejunum.....	32.	17.5 lbs. (905 mm.)	43 lbs. (2223 mm.)	28.2 lbs. (1458 mm.)	32 lbs. (1654 mm.) 37 lbs. (1913 mm.)	12	2.6 cm.	3 mm.
Ileum.....	18.	17.5 lbs. (905 mm.)	37.5 lbs. (1939 mm.)	24.8 lbs. (1282 mm.)	21.5 lbs. (1112 mm.) 27.5 lbs. (1422 mm.)	3	2.1 cm.	4 mm.
Caecum.....	12.	8.75 lbs. (452 mm.)	25.0 lbs. (1292 mm.)	16.9 lbs. (874 mm.)			2.0 cm.	

the submucosa. Tears in the mucosa of the stomach were seen to take place usually before the submucosa and muscles tore, at about 2 lbs. (104 mm.) pressure. These tears appeared on the surface of the distended organ as silvery, longitudinal lines. It was also noted that in every case, without exception, the tear was linear, with clean-cut edges, running in the direction of the long axis of the organ; i.e., in the stomach as already noted; in the intestines parallel to the mesenteric attachment, and nearer to it than to the opposite border; and in the oesophagus extending upward in a longitudinal direction.

Pieces of jejunum deprived of muscles and peritoneum, and consisting only of submucosa and mucosa ruptured on the average of 25 trials at 24.25 lbs. (1254 mm.), which is only 14 per cent lower than the average for the entire gut. The lowest figure in this series was 13.5 lbs. (698 mm.), and the highest 49 lbs. (2533 mm.). In seven duplicate tests carried out in seven cats the greatest individual difference was $1\frac{1}{2}$ lbs. (78 mm.),

the lowest being 13.5 lbs. (698 mm.), and the highest 15 lbs. (776 mm.). Similar tests with ileum deprived of muscles and peritoneum gave 18.6 lbs. (962 mm.) as the average of 16 trials. This is only 25 per cent lower than the pressure necessary to rupture the ileum with all the coats intact. The lowest instance was 9 lbs. (465 mm.), and the highest 27.75 lbs. (1435 mm.). Three duplicate tests were made, in which the greatest was in a cat which gave 24.5 lbs. (1267 mm.) at one time and 27.5 lbs. (1422 mm.) at another.

The following table shows the results obtained by rupturing the jejunum and ileum of the cat deprived of muscle layers and peritoneum. It also gives the length of the tear and the distance of the tear from the mesenteric border.

TABLE 2

Small intestine deprived of muscle coats

	NUMBER OF TRIALS	LOWEST PRESSURE PRODUCING RUPTURE	HIGHEST PRESSURE PRODUCING RUPTURE	AVERAGE PRESSURE NECESSARY TO PRODUCE RUPTURE	LOWEST AND HIGHEST PRESSURES IN AN INDIVIDUAL SHOWING GREATEST DIFFERENCE IN DUPLICATE TESTS	NUMBER OF DUPLICATE TESTS MADE	AVERAGE LENGTH OF TEAR	DISTANCE OF TEAR FROM MESENTERIC BORDER
Jejunum without muscles or peritoneum	25.	13.5 lbs. (698 mm.)	49 lbs. (2533 mm.)	24.25 lbs. (1254 mm.)	13.5 lbs. (698 mm.) 15.0 lbs. (776 mm.)	7	3.4 cm.	1.0 mm.
Ileum without muscles or peritoneum	16.	9 lbs. (465 mm.)	27.75 lbs. (1435 mm.)	18.6 lbs. (962 mm.)	24.5 lbs. (1267 mm.) 27.5 lbs. (1422 mm.)	3	2.4 cm.	2.2 mm.

In all cases of the small intestine deprived of muscles the tear was a longitudinal one with clean-cut edges, nearer the mesenteric attachment, and parallel with it. In these cases the mucosa could be seen to tear at relatively low figures, usually at 10 lbs. (517 mm.) or lower. These tears shimmered through the intact submucosa as longitudinal silvery lines, usually more than one in number.

Fourteen times the specimen from the jejunum or ileum used contained a Peyer's Patch, and when this was present the tear

always took place through it at the pressure indicated on the following table. It gives the results of pieces with and without muscles, from the jejunum and ileum respectively.

TABLE 3

Small intestines containing Peyer's Patches

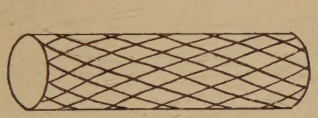
	NUMBER OF TRIALS	LOWEST PRESSURE PRODUCING RUPTURE	HIGHEST PRESSURE PRODUCING RUPTURE	AVERAGE PRESSURE NECESSARY TO PRODUCE RUPTURE	LENGTH OF TEAR	DISTANCE FROM MESENTERIC BORDER
Jejunum, with all coats, through Peyer's Patch.	4.	15.5 lbs. (801 mm.)	24.5 lbs. (1267mm.)	18.5 lbs. (949 mm.)	1.1 cm.	9.0 mm.
Jejunum, without muscles, through Peyer's Patch.	3.	12·lbs. (620 mm.)	13.75 lbs. (711 mm.)	12.9 lbs. (667 mm.)	0.8 cm.	7.25mm.
Ileum, with all coats, through Peyer's Patch.	3.	16 lbs. (827 mm.)	24.25 lbs. (1254mm.)	19.75 lbs. (1021mm.)	1.2 cm.	8.0 mm.
Ileum, without muscles, through Peyer's Patch.	4.	9.5 lbs. (491 mm.)	12 lbs. (620 mm.)	10.8 lbs. (558 mm.)	1.4 cm.	5.0 mm.

As is seen, the rupture takes place at a distance farther away from the mesenteric border than occurs in ordinary specimens, because the Peyer's Patch is situated nearer to the non-mesenteric border. The direction of the tear is again in the direction of the long axis, and, although the tear is linear, its edges are not as clean-cut.

CORRELATION OF ANATOMICAL AND PHYSICAL FINDINGS

The constancy of the longitudinal rupture suggests strongly that the reason for this linear tear in the long axis of the intestine is to be found in some anatomical characteristic of the intestinal architecture. With this idea in view, a study of the fibrous tissues of the gut wall was made in order to ascertain if in the case of the small intestine (the typical hollow cylinder) this contention could be supported.

The small intestine ruptures longitudinally. The tear through the muscle layers is also longitudinal. When one considers that the circular layer of muscle fibres is considerably thicker and stronger than the longitudinal layer, one would expect, from the anatomical relations alone, a circular tear of the muscle coats at least. The submucosa together with the mucosa withstood pressures, as was shown above, only 14 per cent for the jejunum, and 25 per cent for the ileum, less than when all coats were present, indicating that the submucosa is the part of the intestine chiefly concerned in giving strength to the gut. It was found that the main bulk of connective tissue fibres of the submucosa runs spirally around the intestine in both directions, crossing when the gut is moderately distended, so that the acute angle formed by their crossing points in the direction of the long axis of the gut (fig. 1).



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This agrees with the findings of Mall[5] and Clason.[6] When the intestine is distended nearly to the point of rupture these fibres tend to run more nearly transversely (fig. 2), cross at right angles, and many fibres at angles greater than a right angle.

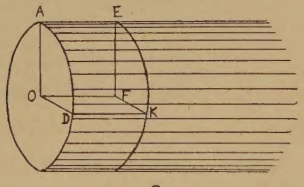
Hence the fibres which in the gut ordinarily run more nearly longitudinally, in the greatly distended gut assume a more nearly circular direction. This, as will be shown, is an attempt to re-enforce the point where the greatest pressure is to be exerted, the reënforcement being taken from the point where the danger from rupture is naturally less according to the laws of physics. Besides this main bulk of coarse connective tissue fibres there is a layer of somewhat finer fibres running longitudinally among the muscle fibres of the longitudinal layer of the muscularis

[5] F. Mall. A Study of the Intestinal Contraction. The Johns Hopkins Hospital Reports, vol. 1, 1896, p. 41.

[6] Clason. Upsal. Lakareförhandl 7. Hofmann-Schwalbe, 1872, p. 182.

mucosae. The muscularis mucosae in the jejunum of the cat is equal to about $\frac{1}{8}$ to $\frac{1}{4}$ of the total thickness of the submucosa. The connective tissue fibres, as can be seen in histological preparations stained for connective tissue, according to Mallory, make up at least one-half of the total bulk of this layer of the muscularis mucosae. The circular layer of muscularis mucosae is much thinner, and contains scarcely any connective tissue fibres.

Naturally, with the anatomic findings favoring a transverse tear, other factors must be considered to account for the constant longitudinal rupture. A consideration of the laws of physics involved in the bursting of hollow cylinders may aid in finding the explanation. The force tending to produce a longitudinal tear as compared with the force tending to produce a transverse tear in a hollow closed cylinder under internal pressure must be considered.



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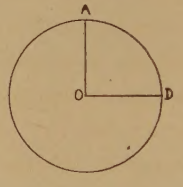

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Let figure 3 represent diagrammatically such a hollow cylinder, and figure 4 a cross-section of the cylinder.

If the radius of the circle O, the end of the cylinder, is equal to one (1), then the area of the circle $\pi R^2 = \pi$.

If p = pressure per unit of area, then $p\pi$ = total pressure on the surface of the circle, or the end of the cylinder.

This force is transmitted to the longitudinal fibres, along the circumference of the circle.

The circumference $= 2\pi R = 2\pi$. Then per unit of circumference we have $\frac{p\pi}{2\pi} = \frac{p}{2}$; which is equal to the force acting in a longitudinal direction on the longitudinal fibres, and tending to produce a circular tear.

If the circumference of the circle O represents a circular fibre, then the force tending to burst it at A is equal to the force acting on the arc AD, at right angles to AO.

Or, continuing AO as a plane AOFE running through the centre of the cylinder, and OD as a plane ODKF at right angles to the first plane, then the pressure on AOFE is equal to the area of AOFE multiplied by p. If AE and OF = AO, which is equal to 1, the pressure on AOFE = p.

This means that the force transmitted to the circular fibres along AE, a unit of length longitudinally, and tending to produce a longitudinal tear is twice as great as the force p/2, acting on a unit of circumference on the longitudinal fibres and tending to produce a circular tear.

The stomach, being more or less of a cone-shaped organ, will also have its tears longitudinal, as will the oesophagus, a cylindrical organ.

Also, the force tending to produce a longitudinal tear along the side of a cylinder which is flattened tends to become less than the force acting along the side with the curve of the smaller radius; so that those experiments mentioned above, where ruptures had been produced by striking distended organs, are not identical with those in which bursting was allowed to take place by increasing the internal pressure alone.

SUMMARY

It has been pointed out that the mucosa of the stomach and that of the intestines ruptured at pressures below those of rupture for submucosa and muscle layers of the stomach and intestines respectively. This would indicate that the mucosa is of slight importance in giving strength to the organs. That the mucosa of the stomach, which is ordinarily thrown into many folds (rugae), indicating a considerable reserve for dilatation, should reach its elastic limit so soon, and tear, is therefore somewhat remarkable. The observation agrees with that of Key-Aberg who said that the tear in the stomach began first in the mucosa. The peritoneum, a very thin and delicate layer of

tissue covering the organs, is also practically unimportant in giving strength to the viscera. The two chief tissues giving strength to the hollow viscera, are, then, the muscle tissue, and the connective tissue of the submucosa. Of these, the pressures for producing ruptures of the submucosa are only 14 per cent for jejunum, and 25 per cent for ileum, less than for the intact intestine. Although there was seen to be considerable variation in the strength of the intestines with and without muscles in different cats, still in the same individual the strength was quite uniform for several specimens obtained from the same regions of the small intestine.

Compared with the other parts of the gastro-intestinal tract, the bursting point of the stomach is remarkably low. That it bursts on or near the greater or lesser curvatures would suggest that either these are anatomical weak places, or that the greater and lesser curvatures are curves of smaller radius than the sides of the distended stomach, and that the tear takes place there because of the physical laws.

Similarly, the tear occurring in the intestines on or near the mesenteric border suggests the same two possibilities. Here, also, no direct observations were made. The figures obtained for the oesophagus were relatively high, and for the small intestine actually much higher than the few cases mentioned in the literature in experiments performed on the dog and the pig. Whether this is due to a difference in the freshness of the tissue used, or to an actual difference in the species of animal, has not yet been determined.

The caecum, another greatly dilatable organ, ruptured at figures lower than those for the small intestine.

Finally, in all the hollow organs tried the tear took place through all the coats, extending in the direction of the long axis of the organ. This agrees with all previous observations. It is explainable, as was seen, not on anatomical grounds, but on the physical phenomenon that in a hollow cylinder (when other things are equal), the internal force tending to produce longitudinal tears is twice as great as that tending to produce circular tears.

CONCLUSIONS

1. The following pressures were found necessary to produce bursting ruptures in the following hollow organs of the cat:

a. Oesophagus	17.75 lbs. per sq. inch	(911 mm. Hg.)
b. Stomach	3.7 lbs. per sq. inch	(193 mm. Hg.)
c. Jejunum	28.2 lbs. per sq. inch	(1458 mm. Hg.)
d. Jejunum, without muscle coats	24.25 lbs. per sq. inch	(1254 mm. Hg.)
e. Ileum	24.8 lbs. per sq. inch	(1282 mm. Hg.)
f. Ileum, without muscle coats	18.6 lbs. per sq. inch	(962 mm. Hg.)
g. Caecum	16.9 lbs. per sq. inch	(874 mm. Hg.)

2. Although the bursting pressures vary considerably for the small intestines of different cats, the bursting pressures in the same individual are quite uniform.

3. The tear produced by bursting is linear and runs in the long axis of the organ. This phenomenon is explainable, not on anatomical, but on physical grounds.

4. The submucosa is the layer chiefly concerned in giving strength to the alimentary tract.

DIRECT UNION BETWEEN ADRENALS AND KIDNEYS (SUBCAPSULAR LOCATION OF ADRENALS)

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FIVE FIGURES

There is a rare condition in which the adrenals lie in part or entirely in direct apposition with the kidney parenchyma with no intervening capsule. It is the so-called subcapsular location of the adrenals. For some reason it has been very generally overlooked both by anatomists and pathologists. It is, however, of importance not merely as a developmental error but, from the pathologist's standpoint, because of its bearing upon the origin of the so-called hypernephromata of the kidney, a question still under discussion. No reference to this condition has been found in any of the modern text-books of pathology, such as Aschoff, Ribbert, Adami and others, nor in any of the works on anatomy which I have consulted. Orth in his Pathologische Anatomie ('93) says "Only seldom is the location of the adrenals in gross changed (from normal). One case is known where the right suprarenal was located at the hilum of the kidney above the renal artery." He makes no reference to its subcapsular location. Broman[1] in his work on the normal and abnormal development of man does not mention the condition. Also in special works on the kidneys and adrenals this abnormality is commonly overlooked. Thus Kelly and Burnam[2] state that in children there is a strong attachment between the kidney and adrenal gland which is less marked in the adult. They make no mention of a direct union between the two organs. On the other hand, Dock[3] in describing the abnormalities of the adrenals says

[1] Broman. Normale u. abnorme Entwicklung des Menschen, 1911.
[2] Kelly and Burnam. Diseases of the kidneys, ureters and bladder, 1914.
[3] Dock. Osler's Modern Medicine, 6, p. 355.

"Pilliet found the right adrenal under the fibrous capsule of the kidney" but he gives no references and mentions no other reported cases. Borst[4] describes the condition briefly as follows, "In certain cases the suprarenal lies entirely or in part close upon the kidney, enclosed in the kidney capsule. (Kelly, Grawitz, Klebs, Ulrich.) Frequently the condition is bilateral and the suprarenal is intimately attached to the upper pole of the kidney as a thin plate."

From this it is evident that the subcapsular location of the adrenal body has been recorded occasionally for years. Grawitz[5] in his second paper on the hypernephromata referred to it. He says that an entire adrenal may be found in the capsule of the kidney or in the kidney cortex, as well as smaller or larger portions of the adrenal. In his earlier paper of the year before ('83) on the same subject he makes no mention of the entire organ being thus displaced. Somewhat earlier than this ('76) Klebs[6] described what is probably a case of this subcapsular location. This was in a well developed man, 20 years old, who died of pneumonia. In place of the adrenals, each kidney was covered over by a thin, yellowish, caplike plate which was beneath the kidney capsule. Microscopically it showed all the elements of the adrenal cortex. At the usual site of the adrenals there was no trace of these. This is the earliest reported case which I have found, although a more careful search might disclose others even earlier. Later Ulrich[7] and Kelly[8] and others reported similar cases. In an excellent summary of the work on the Adrenal Cortex, Its Rests and Tumors (with a good bibliography) by Glynn[9] the only cases of subcapsular location of the adrenal referred to, six in number, are from German authors.

In order to determine the frequency with which the partial or complete subcapsular location of the adrenals has been ob-

[4] Borst. Die Lehre v. d. Geschwülsten, p. 789, 1902.

[5] Grawitz. Archiv. f. klin. Chirurgie, 1884, 30, p. 325.

[6] Klebs. Handbuch d. path. Anatomie, Bd. 1, abt. 2, p. 567, 1876.

[7] Ulrich. Anatomische Untersuchungen uber ganz und partiell verlagerte u. accessorische Nebennieren etc. Zeigler's Beitrage z. path. Anatomie, 1895, 18, p. 589.

[8] Kelly. Zeigler's Beiträge z. path. Anatomie, etc., 1898, 23, p. 293.

[9] Glynn. Quarterly Journal of Medicine, 1912, v, p. 157.

served in this country, I communicated some three years ago, after studying the case reported below, with ten other pathologists[10] who have had opportunity to study a considerable abundance of autopsy material. I take this opportunity to again thank them for giving me the results of their experience. Seven of the ten replied that they had never met with the condition. One other could recall clearly only one case of the kind, while two, Professor Le Count of the University of Chicago and Dr. John H. Larkin of New York, had twice found the adrenal in the subcapsular 'ocation. The former stated that in both of his cases the condition was bilateral. It is quite evident that this condition is among the rare abnormalities involving the adrenals and kidneys.

Aside from being of such rarity as to make it of interest, this abnormal location of the adrenal is of indirect importance in considering the etiology of the so-called hypernephromata of the kidney. As is well known the origin of these tumors from adrenal rests in the kidney, which had apparently been fully determined by Grawitz and confirmed by others, has more recently been seriously questioned. This has been based in part upon the histological differences between the tumors of the kidney and the adenomata found in the adrenal itself, in part upon what may be termed the functional or metabolic differences between these renal tumors and the adenomata of the adrenals, and lastly upon studies of the embryological development of the adrenals and kidneys.

From a histological study of the development of so-called hypernephromata of the kidney, Sudeck[11] as early as 1893 held that there was no connection between these tumors and adrenal rests, but that the former were derived from the tubular epithelium itself. Stoerk[12] after the study of a large number of tumors of this type reached a similar conclusion, namely, that they are of

[10] Drs. W. T. Councilman and F. B. Mallory, of Boston; Drs. T. M. Prudden, James Ewing and J. H. Larkin, of New York; Drs. Ludwig Hektoen and E. R. Le Count, of Chicago; Drs. W. M. L. Coplin and Joseph McFarland, of Philadelphia, and Dr. Horst Oertel of Montreal.

[11] Sudeck. Virchow's Archiv, 1893, 133, p. 407.

[12] Stoerk. Beit. z. path. Anat. u. z. allg. Pathologie, 1908, 42, 393.

renal origin. This was based in part upon the location of these tumors, aways in the kidney, never in the adrenal itself nor in the liver, where so-called adrenal rests are so common; in part upon the histological study of the tumors; and, in part, upon the age at which they most commonly appear, chiefly after middle life, unlike tumors from cell rests which have a tendency to appear in early life.

A further careful study of the subject was made by Wilson and Willis[13] based not only upon the histological examination of 48 tumors of this type but also upon the embryological development of the suprarenal and kidney as shown in serial sections from 26 swine embryos and from 43 human embryos. Their conclusions from this study, namely, that the so-called hypernephromata are not of adrenal origin, are evidently based quite largely upon the complete separation at all periods of development between the adrenal and kidney. They say

> The kidney and the adrenal come into final apposition only by the gradual atrophy of the Wolffian body, and between the two organs there is always interposed the mass of fibrous tissue which represents the stroma of the atrophic Wolffian body. Long before the two organs come into any close anatomic relationship with each other, each has formed a distinct and well marked capsule, which is greatly augmented between the surfaces of the two organs which are directed towards each other, by this dense mass of Wolffian-derived fibrous tissue Indeed it is difficult to conceive how any portion of the adrenal cortex can, during the process of embryological development, become imbedded within the kindey parenchyma without showing between its structure and that of the renal cortex, three distinct laminae of fibrous tissue,—the first derived from its own cortex, the second from the remains of the Wolffian body, and the third from the kidney capsule.

And further,

> From what has been stated above, suprarenal inclusions within the kidney parenchyma must be exceedingly rare, if ever present. The only instances of conjugation of suprarenal and kidney tissue which I have ever seen were masses of suprarenal tissue which were attached to the kidney, projecting above its surface, and invariably separated from the kidney parenchyma by a thick capsule, which not only stripped readily from the kidney, but was also separable into a number of laminae.

[13] Wilson and Willis. Journal of Medical Research, 1911, 24, p. 73.

This necessary separation of the suprarenal gland from the kidney by a thick capsule of connective tissue appears to be one of the main reasons derived from their embryological study for concluding that the islands from which these tumors appear to arise may not be adrenal in origin. It is only upon this one phase of the subject, namely, the possible intermingling of suprarenal and kidney tissue without any separation by a definite capsule, that this abnormal union between suprarenal body and kidney, the subcapsular location of the adrenals, has a bearing.

In this condition the adrenal lies in part or entirely in direct apposition, on its under surface, with the kidney tissue, with in part at least, no more intervening connective tissue than there is between the kidney tubules of the normal kidney cortex.

The case of this kind which I had an opportunity to study was one in which each adrenal was directly in apposition with kidney parenchyma, extending down for a short distance into this. This direct apposition of adrenal and kidney did not involve the whole but only a part of each adrenal on its under surface. This condition as here described was found at autopsy in a coroner's case. Nothing was known of the previous history except that the person has been an alcoholic. The general autopsy findings were unimportant. The body was that of a small woman, 5 feet tall. She was about 30 years old. Aside from certain superficial bruises, the gross pathological findings were bronchopneumonia, obliteration of the pleural cavities by old adhesions, chronic gastritis, suprarenals on each side adherent to the kidney, and a slight degree of chronic interstitial nephritis. No other developmental errors besides those of the adrenals and kidneys were found. No careful search was made for adrenal rests in other structures than the kidneys but a superficial examination did not show any. The microscopic examination of the various tissues, aside from the suprarenal bodies and kidneys, requires no discussion as it merely confirmed the gross appearances.

On each side, the adrenal was found unusually adherent to the kidney. This was more marked on the right side than on the left. The right adrenal extended from the upper end of the kidney down over its anterior surface for about 4 cm. This

Fig. 1 Right kidney and adrenal, showing direct union between the two with extension of the adrenal down into kidney parenchyma.

Fig. 2 Shows lack of capsule between adrenal and kidney, with, in places, upward extension of small portions of kidney tissue into adrenal.

suprarenal was $4\frac{1}{2}$ cm. vertically by 5 cm. wide at its widest part. The upper third of the adrenal was separated from the kidney by areolar tissue. Below this it was firmly adherent to the kidney except for a few millimeters along its inner edge. A vertical section through the adrenal and kidney in this area not only showed in gross no connective tissue capsule separating the two, but in places the adrenal tissue extended down into the cortex of the kidney for a greater or less distance, the deepest extension being about 1 cm. (fig. 1). This adrenal at its upper part, where free from the kidney, was of the usual thickness and appearance. Below this it extended as a thin plate over the upper front part of the kidney. Its thickness here in general was only 1 to $1\frac{1}{2}$ mm. The darker color of the medullary portion in contrast to the cortex could be readily made out, though less marked than at the thicker portion at the upper part of the gland.

The left adrenal was also adherent to the kidney though to a much less extent than was the right one. It was not much larger than usual and was free from the kidney except for an area about $2\frac{1}{2}$ cm. by $1\frac{1}{2}$ cm. at its widest part, where it appeared as a thin plate, adherent to the front part of the upper pole of the kidney, and extending a very short distance into the kidney substance in places. No capsule separating adrenal and kidney could be made out here.

Microscopic examination of sections of the adrenals where they were free from the kidney show nothing unusual. But where they lay as a thin plate attached to the kidney several departures from normal are seen (figs. 2 to 5). Both cortical and medullary portions are present. The part of the adrenals which in gross showed no evidence of a connective tissue capsule between it and the kidney are seen in general to lie directly against the kidney cortex with no connective tissue intervening. This is not so in all places, as here and there a distinct band of connective tissue can be made out separating the two. This is also true of the extensions of the adrenal down into the cortex of the kidney. The adrenal and kidney parenchyma are, as a rule, in direct apposition but in places there is a distinct microscopic band of connective tissue between them. It is noticeable that

in those portions where adrenal and kidney are in direct apposition with no connective tissue separating them, the glomerular layer of the adrenal is lacking. On the other hand, where there is a distinct layer of connective tissue separating them, this glomerular layer is generally well made out. The medullary portion is also present in the epinephritic adrenal plates, and in

Fig. 3 Adrenal (above) and kidney (below) in direct apposition.

the largest downward extension of the right adrenal into the kidney cortex the medullary portion also extends down a short distance. In general, the line of union of the adrenal plate and the underlying kidney is readily made out and is fairly smooth. In places there are extensions of kidney tubules up into the adrenal. That these are kidney tubules can be made out here and there by

finding direct extension of these from the kidney cortex into the adrenal. Similar structures found in the adrenal as far up as the medullary portion are probably from the same source. They consist of somewhat dilated glandular structures lined with a layer of cuboidal epithelium and often containing a hyaline sub-

Fig. 4 Area showing thin layer of connective tissue between kidney and adrenal. The glomerular layer of the adrenal is here seen, but is lacking in figure 3.

stance, staining red by the hematoxylin-eosin method. Where portions of the adrenal extend downward into the kidney, the line of separation is in places very irregular with intermingling of kidney structures and adrenal tissue (fig. 5).

In studying the effect of the direct apposition of suprarenal cells upon the kidney cells, none could be made out. The cells

of kidney tubules lying directly against adrenal cells, with no more intervening connective tissue than is found between the tubules of the kidney cortex, appear as normal as elsewhere. They are not compressed by the adrenal tissue. Occasionally at the line of union a tubule may be filled with pale staining cells and

Fig. 5 Intermingling of adrenal and kidney parenchyma.

some doubt is felt as to whether one is here dealing with adrenal or kidney cells. This is, however, the exception.

That this unusual union of kidneys and adrenals is to be explained as a developmental error, and not in any sense as a beginning tumor growth or the result of an inflammatory process, appears to be self-evident. Where the adrenal lies as a thin plate upon the kidney, the thickness from medullary portion to

kidney is no greater than from the medullary portion to the upper border of cortex. And where there is a downward extension of adrenal into the kidney cortex there is no compression capsule of connective tissue to indicate a disappearance of kidney cells. This subcapsular location of the adrenal has in most of the reported cases, as in this one, been bilateral.

Although the condition is very rare, its occurrence at times is not in keeping with the conclusions of Wilson and Willis that the adrenal and kidney are necessarily at all times separated by distinct layers of connective tissue. It gives definite evidence of the possibility of portions of the adrenals becoming imbedded in the kidney parenchyma as claimed by Grawitz as the basis of his theory of the origin of the hypernephromata.

THE VASCULARIZATION OF THE EMBRYONIC BODY OF HYBRID TELEOSTS WITHOUT CIRCULATION

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FIFTEEN FIGURES

Within recent times it has been shown that certain unusual conditions may be imposed upon the developing vertebrate embryo which give a clue to the process by which such an embryo would normally come into possession of its blood vascular system. These unusual conditions have fallen, so far, into two categories: unusual mechanical relations with reference to the extra embryonic blastoderm, and chemically altered environment at an early stage of ontogeny.

Accounts of these unusual mechanical relations, consisting of partial separation of the embryonic body from the yolk-sac blastoderm, may be found in the writings of Hahn,[1] Miller and McWhorter,[2] who obtained blood-vessels on the operated side of partially excised embryos. In view of the fact that the results of these observers were not universally regarded as adequate proof of the local origin of the endothelium which developed under these experimental conditions, such experiments were extended further to a complete separation of the intraembryonic tissue from the yolk-sac blastoderm in the experiments of Reagan, who succeeded in obtaining blood-vessels in completely isolated portions of embryonic bodies. In addition to the advantage of excluding all possibility of an invasion of the intraembryonic tissue by yolk-sac 'Angioblast,' this latter work was based on mate-

[1] Hahn, H. Archiv für Entw., Bd. 24, 1907.

[2] Miller, A. M. and McWhorter, J. E. Anat. Rec., vol. 9, no. 1, 1915.

rial, the actual condition of the extraembryonic portion of which could be definitely determined; the extraembryonic blastoderm was removed at the time of operation so that the exact status of vascular development could be ascertained.

In his excellent monograph on the early development of the vascular tissue in the cat, Schulte[3] not only furnishes conclusive morphological evidence for the local origin of vascular endothelium, but gives a most comprehensive résumé of the two opposing theories of vascular development and the facts upon which each finds its support. On page 22 he reviews the results of Loeb[4] on the inhibited circulation in chemically treated and hybrid teleost embryos, pointing to the probability that embryos possessed of beating hearts but devoid of circulation, might have non-continuous vascular anlagen. Since the publication of Schulte's work, it has been shown by Stockard[5] and by Werber[6] that in chemically treated embryos these anlagen arise locally and non-continuously in and from the mesenchyme, the failure of the separate anlagen to coalesce being caused by, or at least correlated with, the general arrest of development which the embryo suffers.

The existence of beating hearts in hybrid teleost embryos lacking circulation, as observed by Loeb, has recently been verified by Newman.[7] No author has yet examined such material for the histogenesis of vascular tissue. Since the external conditions of such embryos resemble those conditions in the chemically treated embryos described by Loeb, Stockard and Werber, it seems reasonable to suppose that a similarity of internal conditions may prevail. If this be true, it is evident that a study of hybrid material affords promise of another link in the chain of evidence, the significance of which points in no uncertain manner toward the local origin theory of endothelial development.

[3] Schulte, H. von W. Memoirs of The Wistar Institute of Anatomy and Biology, no. 3.

[4] Loeb, J. Pop. Sci. Mo., vol. 80, p. 5.

[5] Stockard, C. R. Proc. Am. Assn. Anatomists, Anat. Rec., vol. 9, no. 1.

[6] Werber, E. I. Anat. Rec., vol. 9, no. 7.

[7] Newman, H. H. Journ. Exp. Zoöl., vol. 18, no. 4.

Of the several crosses tried, the most favorable seems to be that of Scomber scombrus × ♂ Fundulus heteroclitus ♀. About 30 per cent of the individuals resulting from this cross developed oedematous pericardia and string-like hearts. At the age of five days, such hearts showed little pulsation and gave no evidence of a blood content. In the course of an additional four or five days, more or less marked cardiac contractions could be observed. During this time there occasionally developed in the immediate region of, or as a part of, the distal (venous) end of the heart a large blood-island ('blood-island' in the sense in which Stockard uses the term), the blood contents of which attained a bright color. Such a blood-island always resembled closely those found on the yolk ventral to the tail in many other embryos. These blood-cells were evidently of local origin since they often arose before true blood cells could, by most careful observation, be found in the embryonic axis or on any other part of the yolk-sac; certain it seems from the observation of living material, that they were not passively washed in by the movement of body-fluids, either as colorless erythroblasts or as differentiated blood cells. Neither does it seem probable that they could have originated from cells resembling the erythrocyte anlagen to be found in the 'Gefässstrang' of the embryonic axis nor from the erythroblasts on the yolk-sac ventral to the tail, although inference to the contrary would be possible. Morphologically, the immediate anlagen of such blood cells display no signs of capability of active amoeboid movement. If they possess at all the power of movement, it is very slight. As a matter of course, it is certain that the ultimate anlagen of such a blood-island have not always occupied their definite positions as observed after true blood-cells have differentiated. The anlagen of blood-cells do seem, however, to have occupied such a position before they could truly be designated as blood-cells.

The term 'blood-island,' as applied to such aggregates is to be accepted with some caution. In some cases they are not blood-islands at all. In normal development, mesodermal cells wander out or are proliferated out from the body of the embryo, some (destined, if you wish) to form endothelium, some to form pig-

ment cells, and some to form blood-cells In normal development, endothelium is formed rapidly enough and heart pulsations are sufficiently strong that blood-cells are generally though perhaps not always disposed of as rapidly as their differentiation proceeds. In chemically treated embryos, heart pulsations are often reduced to a minimum; endothelial cavities fail to unite and the anlagen of blood and endothelial cells congregate on the yolk, generally ventral to the tail. In such cases blood-islands might well be called vascular stagnations, or better still, 'blood lacunae' of Werber. Embryos with complete circulation, but reared in more or less stagnant water show yolk-areas (usually accompanied by pigment cells) where blood-cells are certainly of local origin. In some embryos reared in running sea-water no such islands could be found.

When, however, one observes the actual passage of an erythroblast from the intermediate cell-mass to a region on the yolk-sac ventral to the tail, either by the motion of body-fluids or by independent amoeboid movement—if such a power be possessed by an erythroblast—he need not conclude that all the erythroblasts of that region reached there secondarily from that source by this means. It is conceivable that adjacent morphologically nonspecific intraembryonic mesenchyme not contained in or related to the intermediate cell mass had proliferated into the yolk-sac region and had there given rise to erythroblasts. If there is no such possibility, then so far as the yolk-sac is concerned there is but one blood-island and this is intraembryonic, while the 'yolk islands' are mere accumulated fragmentations or accumulated descendants of the one and only true blood-island—namely, the intermediate cell-mass.

The point which we wish to establish in this connection is the fact that islands of blood on the yolk-sac are not restricted, as Stockard maintained, to the region ventral to the tail; this point may or may not be of great significance with reference to the interpretation of normal ontogeny.

On page 216, Stockard states: "The vascular endothelium never gives rise to blood-cells. So that heart, aorta and vessels of the anterior end of the body, although invariably lined with

endothelium, do not contain a single corpuscle in embryos of any age."

As we shall later demonstrate, embryos which have always been devoid of complete circulation may have red corpuscles in isolated and blindly-ending venous anlagen in the anterior end of the embryo.

If it be admitted that heart pulsations in the absence of complete circulation are sufficient to displace blood corpuscles from their locus of origin, or if we admit that erythroblasts possess properties of active migration—as one might be compelled to assume under the embarrassment of certain difficulties, or observe in the living condition—then we must admit that the emptiness of cardiac and aortic vessels has little significance so far as the potentiality of their endothelium is concerned, and we are again left without positive clue to the possible origin of blood-cells. Since an open endocardium may merge posteriorly into a loose and vacuolated mesenchyme, into which the aortic lumen may also open anteriorly, it is not at all improbable that the hydrostatic pressure of the beating heart through the body-fluid may be sufficient to remove blood-cells from their locus of origin to another region—an occurrence which may occasion confused interpretations somewhat analogous to the ancient conception that the arteries were air tubes. Even body movements and gravity are factors which may, and probably do lead to cell displacement. Such possibilities are at least worthy of consi 'eration.

If to the conditions to be found in hybrid embryos there may be attached any great significance, Stockard's generalizations will not universally hold good for vertebrate ontogeny, or at least for teleost development. There is good reason to believe that they will not even hold good for chemically treated homozygous embryos. In Fundulus embryos obtained from cyanide and butyric acid treatment, the hearts may (rarely it is true) be so crowded with erythrocytes that they can scarcely pulsate even though there has been no completely established circulation. Occasionally, a large red blood-island may be observed in the head region, the erythrocytes of which seem certainly to have had a local origin. At least such blood-islands develop in some

cases in the complete absence of a circulation. In amorphous masses of tissue which sometimes result from chemical treatment or from hybridization, blood-islands develop promiscuously and apparently without regard to organization, if such there be.

Less than 10 per cent of the hybrids resulting from this cross, (Scomber scombrus ♂ × Fundulus heteroclitus ♀), developed complete circulation. All imaginable gradations from short lived masses of embryonic tissue to hatching embryos were obtained. Embryos which hatched did so at the age of twenty-five days.

A review of certain observations on normal teleost development may serve as a point of departure for the interpretation of certain conditions observed in hybrid development. Neglecting certain differences of opinion among these writers, it may be said that according to Oellacher,[8] Swaen,[9] Brachet,[10] Mollier,[11] and others, the vessels of the greater axial portion of non-pelagic teleosts have their inception in or in close relation to a longitudinal column of mesodermal cells situated between chorda and gut. This vascular anlage has a double origin through the coalescence of two more or less massive columnar cell aggregates derived from the median somitic surfaces. In the region of the first few (first five, perhaps) somites, the entire 'Strang,' smaller in diameter than in the posterior region, becomes transformed into dorsal aorta. In the remaining region posterior, characterized by the presence of the pronephric system, the prevascular column has a much greater diameter. Differentiated from its dorsal surface (Aortenstrang), or at least in close relation to that surface, there arises the posterior continuation of the dorsal aorta. The greater ventral portion of the column in this posterior region (Venenstrang) develops into erythrocytes. On the periphery of the Venenstrang, the endothelium of the postcardinal veins becomes differentiated. This peripheral endothelium may form a ramifying plexus which imbibes the erythrocytes (Mollier), or the postcardinal endothelium may arise simply by the flattening of

[8] [9] [10] [11] References to the works of these observers will be found in Hertwig's Handbuch, erster Band, erster Teil, zweite Hälfte, p. 1125 and following.

the peripheral cells of the column while the entire central mass differentiates into erythrocytes.

Felix[12] (p. 353) describes at length the "schicksale des Venenstrang . . . . . die Umwandlung seiner central gelegenen Zellen zu Blutkörperchen, seiner peripher gelegenen Zellen zu Gefässepithelzellen. . . . . ."

That there is little fundamental difference between the endothelium of the arterial and venous elements is indicated by the fact that they arise in connection with a common cell-strand and possess a common lumen at the anterior terminus of the cardinals, the latter vessels emptying into the aorta (Mollier). In hybrid embryos, furthermore, the entire 'Strang' may, and generally does, become surrounded by one endothelial tube, which sometimes by reason of its position and anterior connections appears to be the true dorsal aorta. In such cases, the postcardinals may fail to appear, or as such be represented by small, weakly developed independent vessels situated ventro-laterally which would seem to have arisen out of relation to the central 'Strang.' The proof of this assumption is difficult at present but it seems to be the most logical explanation of the conditions observed (fig. 15). In cases in which the 'Strang' becomes surrounded by one tubular endothelium besides which no separate aortic endothelium can be demonstrated, it might be possible to assume that the entire endothelium is venous and that the aorta had failed to form. Such a circumstance seems improbable from what we know of the normal differentiation of aorta and cardinals. A third possibility would be that the endothelium is mixed venous and arterial, whether it be regarded as differentiated from the 'Strang' itself or from the adjacent mesenchyme. A fourth and most remote possibility might be that the endothelium figured by us is not real endothelium; there is nothing to favor such a supposition.

The vessel which we have designated as aorta may in some cases be that which Stockard regarded as stem-vein of the conjoined postcardinals. In no case have we found an extensive 'stem-

[12] Felix, H. von W. Anat. Hefte, vol. 8, Heft 25, p. 346.

vein' possessed of haemophoric properties coëxisting with an aortic endothelium. On page 126, Stockard states that the blood forming portion of the 'stem-vein' is "behind the anterior portion of the kidney, extending into the tail." The derivative of the Gefässstrang anterior to the kidney would, according to Stockard, probably be interpreted as venous, or at least partly as venous. According to Swaen and Brachet, the most anterior portion of the vascular anlage is exclusively aortic; it is for this reason that we have designated the vascular cavity in question as aortic, reserving the right to suggest to the reader that he substitute the words 'stem-vein' for the word aorta, wherever we have made use of the latter term in descriptions of figures 2 to 13, providing he should feel more strongly inclined toward that interpretation. The point is, we have here an independently differentiated endothelial-lined vessel. We have not made a statement that the dorsal aorta is haemophoric. Certain it is, however, that in the normal ontogeny the venous derivative is predominantly haemophoric.

That we are here dealing with a true aorta is indicated by the fact that the endothelial surrounding the posterior portion of the 'Strang' may be a mere continuation of that surrounding the most anterior portion; the latter portion, in the light of normal development, may well be interpreted as aortic, even though it may in some cases contain erythrocytes. In addition to this great similarity of the 'aorta' of such hybrids to that of others more normally developed in which empty cardinal veins have also developed, apparently at a later time, renders a priori reasonable the assumption that we are here dealing with a true aorta. Be this as it may, we have taken liberty to designate this unusual vessel as aorta. Whatever may be its real nature, it has a local origin independent of all other vascular endothelium. This we regard as the central fact of the present work.

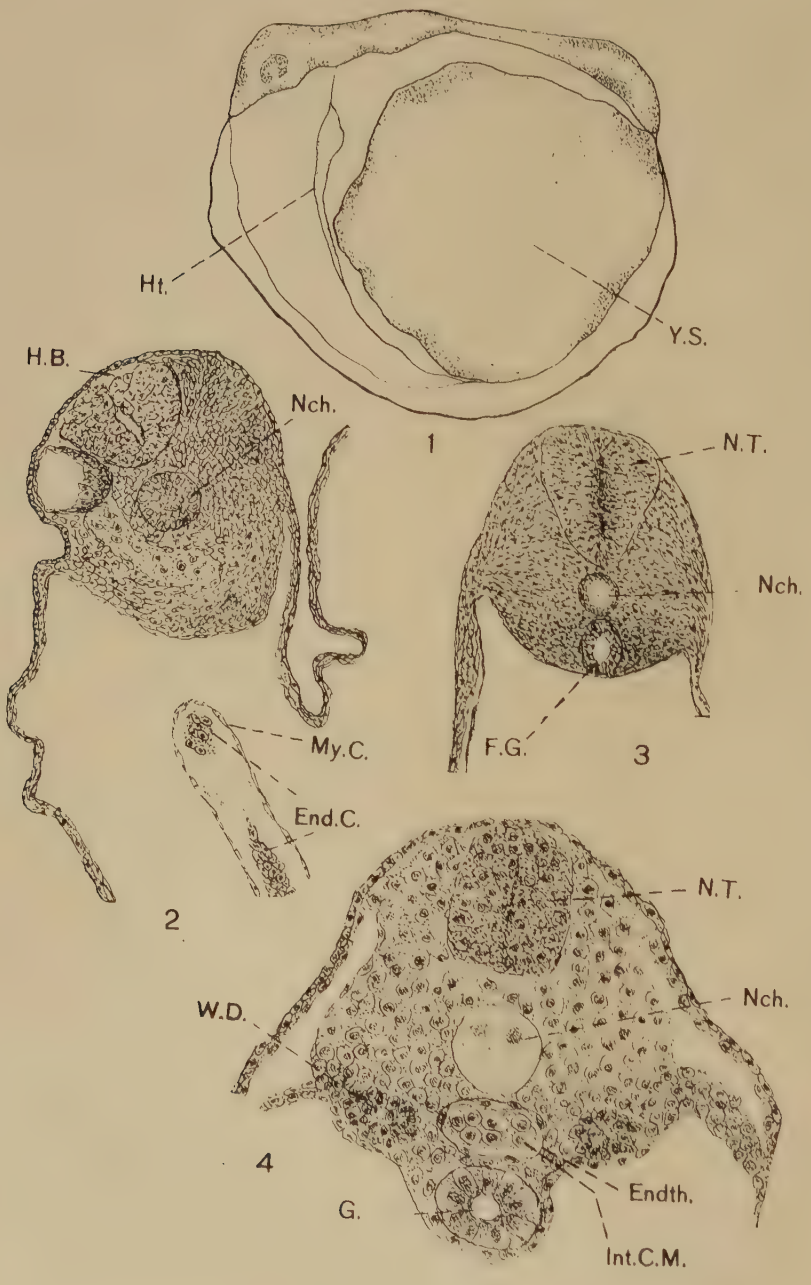
It now remains to consider the actual conditions observed in living embryos, whole-amounts and sections.

Figure 1 represents conditions typical for ten-day hybrids which have never had a circulation. The abnormal distention of the plasma-filled pericardium has stretched the heart out into

a slender tube, closed at either end. Numerous embryos of this sort were obtained. The heart would pulsate and churn its fluid contents up and down. Contractions would sometimes pull the head downward with each pulsation. The reader is referred to Newman's recent work for adequate descriptions of living conditions in such embryos.

Figures 2, 3, and 4 are from cross-sections of a twelve-day hybrid which developed without circulation. The pericardium was distended with a plasma-like fluid so that the heart was stretched out into a long narrow tube between its 'point of origin' on the yolk and its 'point of insertion' on the ventral surface of the embryonic body. The endocardium is seen to be a solid cord of cells. In this section (fig. 2), there is a crescent-shaped lighter area which, from its frequency in sections of such embryos, would seem to be a thinning or vacuolation of the mesenchyme preparatory to a linking of the endocardial tube with the dorsal aortic anlage present considerably farther back in the embryonic body (fig. 4, Endth). Reading back in the series (fig. 3), it will be found that all signs of vascular tissue are absent. The notochord is in close proximity to the gut, so that very little mesenchyme can be found between them. Tracing still further (fig. 4), one encounters an independent and completely isolated vascular anlage in the periphery of which the cells have begun to flatten out to form endothelium. The central cells have acquired an oval or rounded contour; these cells still remain in a more or less syncytial relationship, or at least connected by protoplasmic bridges. They are to be regarded as intermediate in form between indifferent mesoderm and differentiated erythroblasts.

Figures 5, 6, and 7 are cross-sections through the body of a ten-day hybrid. Although this embryo is slightly younger than the one just described, its differentiations have proceeded more rapidly. This is not at all surprising when one considers the fact that the rate of differentiation varies with the degree of normality, which in turn, according to Newman, varies with the extent to which the hybrid displays maternal or paternal characteristics. The heart of this embryo was observed in the liv-


Ht.
Y.S.
1
H.B.
Nch.
My.C.
End.C.
2
N.T.
Nch.
F.G.
3
N.T.
Nch.
W.D.
4
G.
Endth.
Int.C.M.

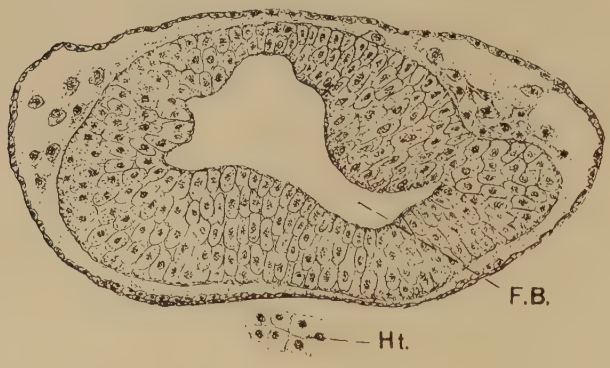
ing condition to be greatly stretched, solid in its distal extremity but hollow near its attachment to the embryonic body. No circulation was ever established. Figure 5 shows a section through the anterior end of this embryo. The heart (Ht.) lies ventral to the head tissue. As the section passes through the more distal portion of the heart, the latter appears as a solid cord of cells. Figure 6 is taken more posteriorly, the plane of section passing through the region where the heart joins the body—that is, through the aortic end. There is a rather indistinct endothelial lining in which may be seen rounded cells which may be transforming into erythroblasts. Tracing posteriorly a few sections as in the preceding figure, one finds (fig. 7) that the endocardium of the heart has ended and no vascular endothelium can be found in the section. The anterior end of the aortic anlage is first encountered about 275μ back of the posterior limit of cardiac endothelium. Figure 8 is a section through the anterior end of the aortic endothelium which is quite distinct, bounding a dorso-ventrally compressed slit-like cavity. Traced anteriorly, this endothelium loses itself in the indifferent mesenchyme. Somites are not differentiated in this embryo, so that besides the somewhat abnormal Wolffian ducts there are no landmarks by which the boundary between pure arterial and mixed arterial

EXPLANATION OF REFERENCE LETTERS

Ht., Heart
Y.S., Yolk-sac
Nch., Notochord
My.C., Myocardium.
End.C., Endocardium.
N.T., Neural tube
W.D., Wolffian duct
F.G., Fore-gut
G., Gut
Endth., Endothelium
In.C.M., Intermediate cell-mass
Pr.C., Pre-cardinal vein
Erth., Erythrocytes
Aort., Aorta
Pec., Pericardium
D.Cv., Duct of Cuvier
Mch.S., Mesenchymatous space
V.Aort., Ventral aorta

Fig. 1 Twelve day hybrid. Scomber scombrus ♂ × Fundulus heteroclitus ♀ (cleared in xylol).

Figs. 2, 3 and 4 Sections through the body axis of a twelve day hybrid (same cross); 2, through the heart (× 180); 3, through the anterior end of the fore-gut (× 180); 4, through the trunk region (× 350).



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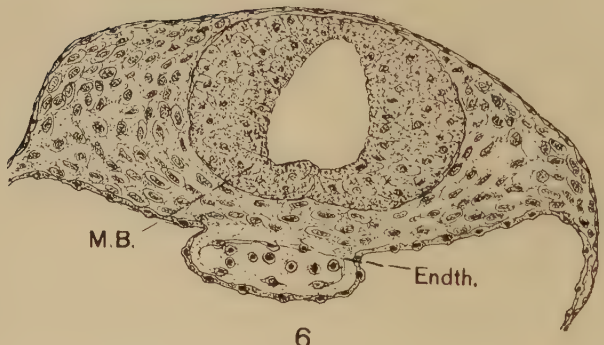

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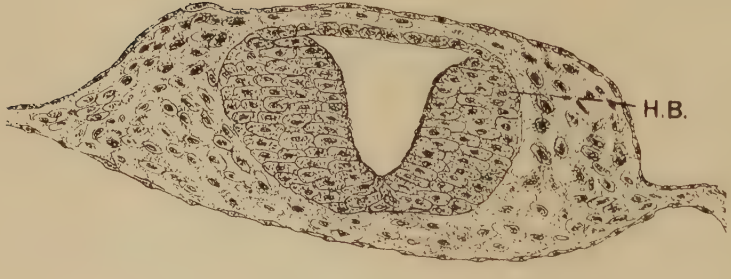
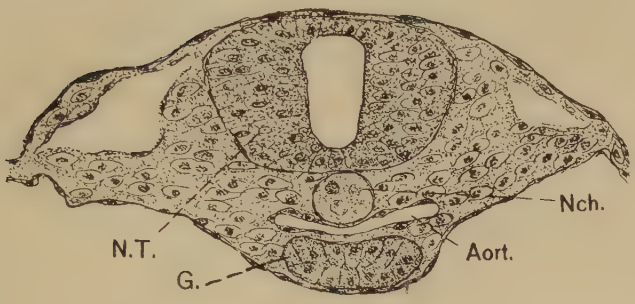


7

Figs. 5 to 12 Sections through the body axis of a ten day hybrid (same cross); 5, through the distal portion of the heart (× 350); 6, through the proximal portion of the heart (× 350); 7, through the hind brain (× 350); 8, 9, 10, through the body axis anterior to the pronephros (× 350); 11, detail of ventral portion of figure 10 (× 700); 12, through the body axis in the region of the pronephros (× 400).



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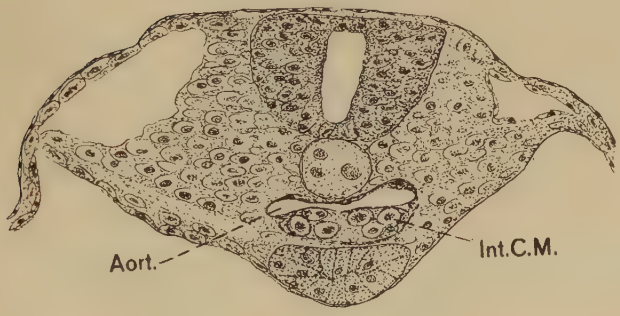

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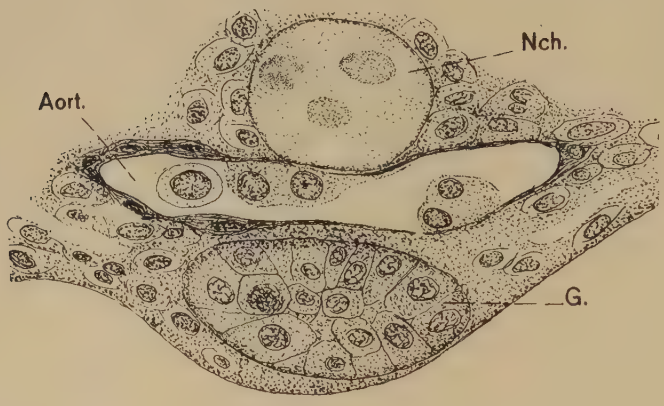

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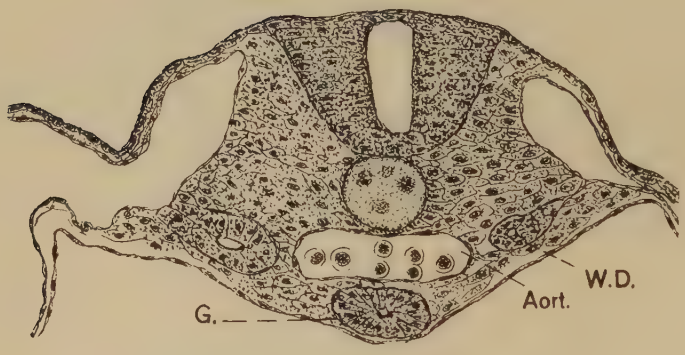


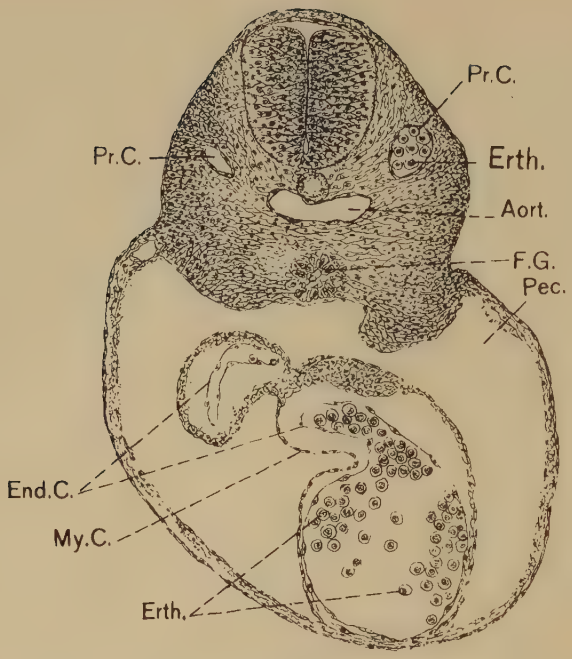
12

and venous portions of the intermediate cell-mass can be definitely located. From the fact that the pronephric tissues is situated a great distance posterior to the plane of this section, we may feel assured that we are here dealing with 'Aortenstrang' somewhere in the region of the most anterior potential somites.[13] Tracing more posteriorly but still anterior to the pronephrogenous region, one finds the same slit-like cavity (fig. 9) situated

[13] The reader is requested to compare our figures 8 and 9 with figures 809 and 810 in Hertwig's Handbuch. Erster Band, erster Teil, zweite Hälfte.

dorsal to a rather darkly staining mass of cells, bounded ventrally by a deeply staining membrane which is continuous with the lateral extremities of the aortic endothelium. This mass of cells might be interpreted as cells of the Venenstrang if the section were not so far anterior to the pronephric region. Tracing still more posteriorly (figs. 10 and 11), one finds continuous with the slit-like aorta, a large vascular lumen containing a few erythrocytes and traces of Strang-cells evidently being separated from the mesodermal column. Their nuclei are large and rounded; their cytoplasm is lightly staining. The nuclei are in a syncytial relationship. The cells are separated from the extra-vascular mesenchyme by a distinct endothelium which is less perfect adjacent to these cells than elsewhere. As before stated, this region is anterior to the pronephric anlage whether the section is through the 'Aortenstrang' alone, or through the anterio-venous portion of the intermediate cell-mass. Figure 12 is a section through the region of the pronephric ducts, a region which should normally contain both arterial and venous endothelium. There is, however, but one vascular cavity (which we designate arbitrarily as aorta) containing free rounded blood cells. Posteriorly, the erythrocytes and the endothelium merge gradually into indifferent mesenchyme where they lose their identities.

Unusually interesting conditions were exhibited by another ten-day hybrid from which figures 13, 14, and 15 are taken. The known history of this embryo lends interest to the conditions which its sections disclose. The embryo never developed a complete circulation, although the heart was almost normal in shape and the pericardium appeared but slightly distended. On the fifth day, the heart was pulsating slowly but rhythmically; its proximal (arterial) portion appeared, so far as could be observed in the living condition, to be an open tube possessed of a continuous lumen. The distal end of the heart appeared to end blindly. At the region of its 'origin' in the yolk-sac, just as the limit of the pericardial cavity, the cardiac anlage was perfectly solid. There could be detected no entrance of cellular elements into the heart, either by active or by passive means. Weak



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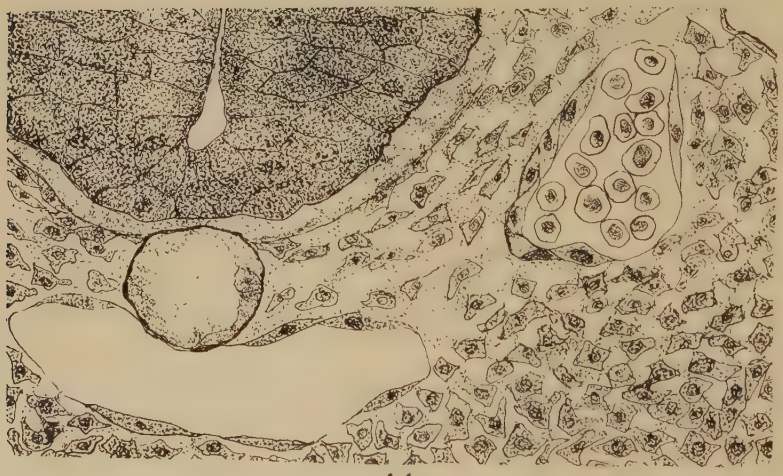

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Fig. 13 Cross section through the body axis of a ten day hybrid (same cross), the plane of section passing through the distal extremity of the heart, showing vacant aorta but blood-containing heart and pre-cardinal (× 200).

Fig. 14 Detail of region of containing pre-cardinal and aorta from the right side of figure 13. Note the emptiness of the aorta and the presence of erythrocytes in the precardinal (× 850).

pulsations continued in this manner, gradually becoming weaker perhaps, until the embryo was killed. The heart itself gradually became more distended and a well defined left-lateral flexure developed. Late on the eighth day, its contents began to display an orange hue. When the embryo was killed and sectioned on the tenth day, it was found that the heart contained numerous erythrocytes. The ventral (venous) end of the heart ended blindly at the pericardial limit, seeming never to have had ventral connection with a vascular cavity or continuous vessel. The endothelium of the heart faded gradually into a loose and vacuolated mesenchyme. The cardiac endothelium did not communicate with aortic endothelium, but the latter merged into the same vacuolated mesenchyme in which the posterior portion of the cardiac endothelium also lost itself. The two defined vascular cavities were well separated. The dorsal aorta was found to be completely devoid of blood cells. Posteriorly the aorta (fig. 15) communicates directly with the postcardinal vein which contains blood cells. Figure 13 shows the precardinals to be haemophoric, as had been previously inferred from observations on the living embryo. The ducts of Cuvier are likewise haemophoric. A reconstruction of this embryo (fig. 15) shows the precardinals terminating blindly in the anterior region. Traced back, they are found to course laterally and discontinuously as the ducts of Cuvier (D. Cv.) and to end blindly on the yolk-sac. In this embryo it is quite conceivable that the action of heart pulsation on the body fluids may have been communicated through the loose mesenchyme to the aortic cavity. *That there was no complete circulation of cellular elements is indicated by the fact that the blood cells never left the cardiac tube to enter the aorta.* Cardiac and aortic cavities never communicated but were separated by a loose mesenchyme. Also the heart had a blind ventral (venous) ending.

This embryo illustrates very well the fact that the *aorta may be very well developed when postcardinals are practically absent.* Thus it is seen that the interpretation of endothelial cavities in these embryos is often exceedingly difficult. For this reason we

Fig. 15 Reconstruction of the vascular system of the embryo from which figure 13 is taken, showing the blind ventral end of the heart, the discontinuity of heart and aorta, the discontinuity of the aorta and of the various venous elements (× 200). A section through the trunk-region might lead one to the erroneous conclusion that it contained a 'stem-vein.'

do not insist upon our interpretation of the 'aorta' in figures 2 to 13.

It is difficult to formulate a possibility by which the erythrocytes contained in the precardinals of this embryo could have reached their present status unless these vessels are haemophoric. The action of the body fluids seems entirely inadequate to account for this phenomenon. In this and in many other instances, the ducts of Cuvier seem surely to be haemophoric channels; sometimes they are preceded by a densely staining 'Strang' of cells resembling the postcardinal 'Venenstrang.'[14]

It might be added that the sub-intestinal veins may in some cases contain erythrocytes, which means either that these vessels are haemophoric[15] or that their contained cells have been washed in from the dorsal aorta. Lacunae of erythrocytes develop in various locations in the head region. Since erythrocytes may develop in practically any region of the body, it is not improbable that continued search may disclose endothelial cavities containing both leucocytes and erythrocytes, *provided that true leucocytes are ever developed in embryos which have never possessed a circulation.*

As before stated, the primary purpose of the present work was to determine whether hybrid material would furnish another basis of proof of the local origin theory of endothelium. When we began the work, nothing was farther from our purpose than to trespass in the field of haematology. If our results do nothing more than stimulate a more minute and exacting study of the possibilities of error in the interpretation of conditions in embryos devoid of complete circulation, they would seem to be worth the time which has been expended on them.

A study of the 'Gefässstrang' of the hybrid teleost convinces us that aortic and postcardinal endothelium stands in no direct cell-lineage with that of the yolk-sac.

[14] This condition is quite striking in crosses between F. heteroclitus ♂ and F. majalis ♀.

[15] The term haemophoric as employed by us denotes the condition of a vessel when it contains red blood cells which were not secondarily and passively carried there by body-fluid in motion.

In conclusion, we wish to thank Professor C. F. W. McClure for his kind interest in our work and for many valuable suggestions. Acknowledgment of indebtedness is also due Professor P. H. Mitchell, Director of the U. S. Bureau of Fisheries, Woods Hole, Massachusetts, for numerous courtesies extended to us in his laboratories.

A FURTHER STUDY OF THE ORIGIN OF BLOOD VASCULAR TISSUES IN CHEMICALLY TREATED TELEOST EMBRYOS, WITH ESPECIAL REFERENCE TO HAEMATOPOESIS IN THE ANTERIOR MESENCHYME AND IN THE HEART

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TWENTY-SIX FIGURES

As first shown by Loeb,[1] certain chemicals are capable of inhibiting, arresting, or preventing complete circulation in embryos of Fundulus heteroclitus. The same phenomena have since been recorded by Stockard[2] and Werber.[3] Stockard's highly interesting study is apparently based on the following postulate: In embryos "in which the blood fails to circulate on account of the fact that the heart is either blind at one end or both, or fails to connect with the veins" (p. 124) it is safe to conclude that any place in which blood-cells are found may be considered their locus of origin, even though in some cases the "embryo develops in a fashion closely approximating the normal when the heart beat is fairly strong." Incidental to this main postulate his work relies on the proposition that tissues devoid of blood-cells under such conditions are proved to be, and always to have been, devoid of haematopoetic potentiality. Thus the demonstration of endothelial-lined cavities which contain no blood-cells demonstrates likewise the inability of all endothelium to produce blood-cells. The absence of blood-cells in the liver disposes of that organ as a blood producer in the bony fishes (p. 125). Another highly interesting observation of Stockard's

[1] Loeb, J. Pop. Sci. Mo., vol. 80, p. 5.

[2] Stockard, C. R. Proc. Am. Assn. Anatomists, Anat. Rec., vol. 9, no. 1.

[3] Werber, E. I. Anat. Rec., vol. 9, no. 7.

(and very important if it is correct) is that "the erythrocytes in Fundulus embryos have . . . . two distinct and limited places of origin, first in the stem vein or conjoined cardinal veins, and second from the blood islands of the yolk sac;" the latter, according to Stockard, are "always on the posterior and ventral yolk surface and do not extend over the anterior surface." Also in these embryos lacking circulation owing to the circumstances above outlined by Stockard "the heart, aorta, and vessels of the anterior end of the body, although invariably lined with endothelium, do not contain a single corpuscle in embryos of any age."

There are, however, possibilities which should be thoroughly considered. Certain it is that heart pulsation in the absence of complete circulation may profoundly change the original and true picture of the actual genetic processes. At present I shall enter into this question no further than to state that I have observed in the living chemically treated embryo the actual passage of a fully differentiated erythrocyte from the intermediate cell-mass to a large and bright red blood-lacuna on the yolk-sac ventral to the tail. I could see no evidence of active amoeboid movement but could observe the oscillation of such cells in the plasma-like fluid which contained them. Such oscillations followed the rhythm of heart pulsation. The heart was open at its arterial end, closed at its venous end so that circulation was incomplete. Concerning the possibility of the active migration of late erythroblasts I can say little from direct observation except that I have never yet been able to detect any such active movement.

I wish at present to consider certain groups of erythrocytes to be observed in a number of cases in the anterior end of living, chemically treated embryos which have always been devoid of circulation. These groups of blood cells are not very large or conspicuously red except in rare cases. They are, I believe, of sufficiently constant occurrence to be of significance for the normal development. Embryos which develop relatively normal eyes and fore-brains sometimes have a tendency to exhibit local and often bi-laterally symmetrical patches or erythrocytes at

an early state of their ontogeny, in the region of the eyes. The greater number of such cell groups which I have observed are located dorsal to and just median to the eyes. Often they may be anterior, posterior, ventral to, or even completely circumscribing the eye, or occupying a space in the optic cup. The time during which they display a content of haemoglobin is usually very short under the most favorable conditions of oxidation. I have quite often had the experience of studying such a cell group, setting the embryo aside for a few hours and reëxamining it again only to find that the red color had entirely disappeared. Occasionally I have observed such a patch of cells on one side of the head dorsal and medial to the eye on that side. After sketching the position of this haematopoetic region I have re-examined such embryos a few hours later, sometimes to find that a similar group of cells had made its appearance on the opposite side and symmetrical with that previously studied and sketched, the group first studied having lost its red color. During such a study all possible efforts were made to detect the active or passive movements of such red colored cells, but careful examination gave no evidence of any sort of movement.

The region between the otic vesicles and also the middle anterior portion of the snout displayed such temporary patches. In still rarer cases groups of blood-cells could, at a later stage of ontogeny, be observed in the mandible and occasionally in practically any position in the mesenchyme of the anterior end of the embryo. Blood was sometimes, though rarely, observed in hearts having solid cord-like venous and arterial extremities, and dilations in their middle portions containing bright red erythrocytes. In some instances there was a series of such dilations (fig. 15).

It is interesting to note that in certain dishes of experimental embryos and at a very definite age, with conditions under which I made my study, many embryos which had been subjected to the same sort of treatment would exhibit a large number of peculiarities in common. It is not yet safe to say that definite substances produce specific results. The tendency is, I believe, for writers on the subject to lump all peculiarities and abnor-

malities into the category of developmental arrest. It is difficult for me to believe, when a given egg is subjected to the action of a protein-precipitant (such as alcohol), or to the action of a fat-solvent (such as butyric acid) that the resulting embryo would be afflicted in precisely the same manner as if, instead, it had at the same time been subjected to the lecithin-precipitant acetone. The relatively great variability of different eggs and the relatively great difference in the effects which a given substance has at different stages of ontogeny are generally sufficient to overshadow whatever specific action a chemical may produce.

It will be sufficient for the present work to figure certain of the conditions found in chemically treated embryos. Let us first consider some of the cases of haematopoesis in the mesenchyme of the anterior end of the embryo. Figures 1 to 10 show sketches of the heads of chemically treated living embryos in which erythrocytes have differentiated locally. In none of these embryos was there a blood circulation. In most cases the hearts were solid, both at their venous and arterial ends, and in some cases solid throughout. The stippled areas represent aggregates of erythrocytes. In all cases in which the embryo was killed at the time when these haematopoetic areas displayed a red color, the blood-cells would stain brightly red with eosin in my modification of Mann's methyl blue-eosin stain.[4] In many cases the area thus observed was not quite coëxtensive with the strongly eosinophilous and haematopoetic region revealed by the stained sections. I interpret this to mean that the developing blood-cells may contain sufficient haemoglobin to stain differentially without having a sufficient amount of that pigment to be discernible in the living embryo. I have never yet found the eosinophilous area to be smaller than the visible red area of the living tissue.

The method of chemical treatment of each embryo here described is given in connection with the explanation of figures. The first eight figures represent dorsal views. Figures 9 and 10 are lateral views showing especially well the distended peri-

[4] Reagan, F. P., Anat. Rec., vol. 8, no. 7.

cardium and string-line hearts. In the embryo represented in figure 1, blood lacunae were observed in the living conditions dorso-medial to the eyes, dorso-posterior to the eye, and a single unpaired median dorsal lacuna was found just beneath the ectoderm in the region slightly anterior to the otic vesicles. The position and nature of these lacunae is shown in section in figures 19, 20, 21, and 22. All these lacunae displaying a bright red color in the living condition were found correspondingly located in the embryo when it was subsequently sectioned and stained. Certain haematopoetic areas could also be found in section which were not visible in the living tissue. The heart of this embryo was, from the beginning, solid at both extremities.

In figure 2 there are seen to be two lacunae, medial and dorsal to the optic cups. The red color disappeared from these regions within four hours after they were discovered. Figures 3 and 4 are sketches of the dorsal cephalic surface of another embryo, the sketch for figure 4 having been made at a stage 14 hours older than that of figure 3. The embryo from which these sketches were made has the following history. At its four-cell stage on July 18 it was placed in a solution of 50 cc. sea-water containing 10 cc. of $\frac{1}{12}$ molecular butyric acid, where it remained for 24 hours. At 10 a.m. July 27, the red lacuna over the left eye was observed and sketched. At 2 a.m. the following morning a red lacuna was observed over the right eye while that over the left eye had lost its color. The embryo was subsequently sectioned. Eosinophilous areas surrounded by endothelium and corresponding respectively to these lacunae were observed in section, their staining capacities being about equal. Figures 5 to 9 show a variety of positions in which similar lacunae were found in various embryos and later verified by sections. In some sets of experiments over 10 per cent of the embryos in a given dish might at a given time display lacunae comparable to these. Unfortunately my attention became attracted to these particular phenomena rather late in the spawning season of Fundulus, so that I am at present unable to offer statistics on their constancy. I am led to believe that if one could observe such experiments in-

cessantly, the embryos found to exhibit these conditions would not compose a small minority.

Figure 10 shows a side view of a rather remarkable embryo. At its four-cell stage it was treated for 24 hours with a mixture of equal parts of 4 per cent alcohol (sea-water solution) and a solution of potassium cyanide made from 2 cc. of $\frac{1}{100}$ per cent KCN in 50 cc. sea-water. The embryo developed without blood circulation. On the twelfth day it exhibited haematopoetic areas conveniently viewed through the translucent otocysts. Within the next three days a single median haematopoetic area was observed in the mandible. These conditions continued for some time; on the twenty-fifth day the sketch for figure 10 was made. Blood cells were then observed scattered over the floor of the pericardium. Within the next five days the red color had practically disappeared from the anterior haematopoetic areas. The embryo was allowed to live in running sea-water until it was forty-three days old; when it was sectioned there were found columnar longitudinal cell-aggregates medial and ventral to each otic vesicle undoubtedly representing precardinal lines. They could be traced discontinuously back to their junction with the postcardinals. The central cells of these columns were very weakly eosinophilous, staining reddish purple rather than bright red as do normal erythrocytes. The surrounding mesenchyme took the usual blue color. The cell column in the mandible, the scattered blood-cells in the floor of the pericardium and those of the posterior axial region stained similarly to those of the precardinal line. Descriptions of sections of this embryo are reserved for a later work.

The conditions in figures 11 and 12 are of interest in that they demonstrate the fact that blood cells may develop on any part of the yolk-surface under conditions in which heart pulsation could not have been a factor in determining their location. In both these embryos the heart has failed to develop. Figure 11 shows an embryo where a small portion of the body axis abuts upon a much dilated pericardium in which no heart is to be found. On the floor of the pericardium near the junction of pericardium and yolk-sac will be seen numerous scattered erythrocytes.

Some have even developed on the inner surface of the pericardium. It is preëminently (though I should not say universally) characteristic of such embryos that the blood cells of the yolk-sac develop in this scattered manner when the heart fails to develop.

In figure 12 we have a condition in which the embryonic body is entirely lacking. The entire yolk-surface is covered with blood cells more or less scattered, but in some regions rather densely aggregated. In the center of the region towards the observer, the blood cell mass is enclosed by endothelium. In some instances in which the embryonic body fails to differentiate, these endothelial haemophoric cavities are relatively numerous and may even join to form intricate plexuses.

The embryo upon which figures 13 and 14 are based is of extraordinary interest. At the four-cell stage this embryo was treated for 20 hours with 3.5 per cent alcohol. On the sixth day there was noticed an elongated blood lacuna on the antero-ventral yolk-surface, very conspicuous for its fiery red color. No other erythrocytes could be found in the yolk-sac or body axis. The heart seemed to be closed or obstructed at the venous end but was otherwise apparently normal. There was no circulation though the heart was pulsating. On the tenth day a decided tremor could be observed in this 'lacuna' as a wave of contraction starting from the end nearer the venous end of the heart and passing to the opposite end. There had also developed a slight flexure, the convexity of which was dorsally directed (fig. 13). At this time the churning movement of the heart could be seen to loosen the closely packed cells in the intermediate cell mass which had begun to acquire a faint orange color. On the fifteenth day I was able to follow certain of these dislocated and rounded erythroblasts as they were dispersed to their later position on the yolk-surface, where they were accumulating in great numbers and apparently multiplying rapidly. I am convinced that those 'blood-islands' so often observed on the yolk-sac ventral to the tail are occasionally of this sort of formation. At a stage earlier than either here described certain observations (which I shall consider at another time in greater detail) were

made on the formation of the endothelium on the yolk-sac. Single mesenchymal cells were observed in active migration. They would align themselves into short solid or hollow cylindrical aggregates of isolated endothelium, many of which joined each other. At the age of ten days, this activated 'lacuna,' accessory heart, or whatever it may have been, was pulsating feebly. On the nineteenth day (fig. 14) it was pulsating seventy times per minute, while the real heart, seemingly normal in shape was pulsating one hundred times per minute. The yolk-sac vessels at this stage were still discontinuous. So long as this embryo was observed, no vessels connected with, or developed immediately adjacent to the accessory blood-filled heart. In later stages the accessory heart gradually increased its pulsation rate when on the twenty-fifth day its rate was 136 pulsations per minute, whereas the actual heart-pulsation went at a rate of 126 times per minute. At this time the vitelline circulation was established. The embryo was killed on the twenty-sixth day when the accessory heart had begun to slow down considerably. I have no explanation to offer for this extraordinary state of affairs. Especially puzzling is the direction of pulsation in the 'accessory heart.' I was unusually fortunate in being able to do my work in a laboratory where I could avail myself of the counsel of many observers—the Marine Biological Laboratory, Woods Hole, Massachusetts. It was with reference to this embryo that I consulted many biologists. At the time when the sketch for figure 14 was made, the embryo was examined by Profs. E. G. Conklin, H. McE. Knower, E. I. Werber, and E. V. Cowdry. At a somewhat later time, when the vitelline circulation had been established, the embryo was examined by Prof. C. R. Stockard and others. I regret very much to be unable to figure sections of this interesting embryo owing to the fact that it became in some way mislaid.

Figures 16, 17 and 18 represent cross sections of a nine-day embryo, which, at the four-cell stage, had been treated with butyric acid. It will be seen that certain mesenchyme cells have been transformed into erythrocytes. This is especially striking in the optic cup (at least the invaginated portion of the brain

tissue on the reader's left) in figures 16 and 17. Likewise there is a region of haematopoesis dorsal to the optic tissue as shown on the right side of figure 17. These erythrocyte lacunae are completely isolated, having absolutely no connection with the conjoined cardinals. They were observed in the living embryo as red patches of blood tissue. Anteriorly the heart is solid (fig. 17); more posteriorly it possesses a small lumen (fig. 16). The heart again becomes solid at its arterial 'insertion' in the embryonic body.

In figure 18 the symmetrically situated precardinals are conspicuously haemophoric. The cells in these vessels were observed in the living condition to possess a content of haemoglobin; nevertheless the cells are so closely packed that their contours are not rounded but present plane surfaces. I have often observed conditions similar to this—that is, a haemoglobin content in cells not yet typically erythrocyte in shape, owing to their closely crowded condition. These erythroblasts are surrounded by endothelium.

This embryo is, I think, of very great importance. Owing to the curious eye-formation it attracted my attention more than most other embryos which I studied. I feel quite certain that the erythrocytes in the anterior end of this embryo were not carried there by any sort of circulation or movement of body-fluids. I kept close observation on the yolk-sac and in observing the living condition never found a single moving or stationary erythrocyte or any formation of endothelium in the anterior hemisphere of the yolk-sac. Figure 16 shows a section in which the yolk-sac is included. In this section there is not a sign or trace of vascular tissue. The same is true of all sections of the yolk-sac anterior to this, and of many which are posterior. *In this embryo the solid venous end of the heart ends blindly on the yolk-sac, the anterior portion of which is, and always has been, devoid of any sort of vascular tissue, endothelial or corpuscular, yet the anterior vessels do contain erythrocytes and the anterior mesenchyme is haematopoetic.*

It may be profitable to divert our attention long enough to consider the eye abnormalities in this embryo. On the left side

of figure 16 there is what I would interpret as an optic cup with more or less distinct choroid and retina. The space normally occupied by a lens seems to have been filled with haematopoetic mesenchyme which gave rise to a blood lacuna that is more (fig. 16) or less (fig. 17) completely surrounded by endothelium. It was this lacuna which induced me to continue unusually careful study of the living embryo. The plane of section in figure 16 is almost tangential to the more dorsal lens of the opposite side. On the right side of figure 17 it will be seen that there are two optic cups and two lenses, each of the latter appearing to be normal in size. It is not my place to advance an explanation of this ophthalmic dilemma; I merely present the facts as of possible importance to those engaged in the highly interesting controversy over the explanation of cyclopia. If, however, both eyes originate and diverge from a median anlage, and if cyclopia is an expression of a weakening of this anlage by toxic substances, as Stockard maintains, the conditions here observed could, so far as I can see, be explained only by assuming that the anlage, instead of becoming stupefied or stunted, had been stimulated to an unusually great activity, rivaled only by the conditions in Werber's figure 8 (loc. cit).

In figures 19 and 20 we have sections of certain of the haemopoetic areas sketched grossly in figure 1. Figure 19 is taken from a section just posterior to the optic vesicles and shows the anterior limits of the two areas dorsal and posterior to these optic vesicles. The blood-producing regions are roughly indicated in this figure by the areas containing the large black dots; the two small blood-producing areas on the right side of figure 19 are shown at a considerably higher magnification in figure 21. The erythrocytes, represented by the cells with un-stippled cytoplasm, are large and rounded. Their large nuclei are very strongly eosinophilous while their peripheral cytoplasm remains almost colorless. Judging from their morphological characterictics and microchemical reaction they are absolutely identical with the undoubted erythrocytes of the postcardinals, of the yolk-sac, and with those of local origin in figure 24. The adjacent mesenchyme cells have very small nuclei and take a bluish purple stain. Certain of these

mesenchyme cells seem to be transforming into erythrocytes. Their nuclei have become larger, their staining reaction less basophilic, and their contour is becoming more rounded. Not all of the strongly eosinophilous cells have yet become rounded. Especially in the lower area has the cavity of a potential blood-vessel made its appearance; the mesenchyme cells around this cavity are relatively more crowded than those more distant from the vessel, and some of them have begun slightly to flatten out to form endothelium.

Figure 20 represents roughly the position of the posterior dorso-median blood-cell group of figure 1. This cell-group is shown in accurate detail in figure 22. The differentiating erythrocytes are quite strongly eosinophilous, but do not have rounded contours by reason of their closely crowded quarters. Their status is similar to that of the erythrocytes in the precardinals in figure 18, and of erythroblasts often seen in the intermediate cell mass.

Let us now consider the possibility of haematopoesis in the heart. As has already been noted, blood-cells may appear in the dilated portion of a spindle-shaped heart which has always been solid at both ends, and erythrocytes were observed to develop locally in the accessory heart of figures 13 and 14. The possible anlagen of such cells might be endothelial, myocardial, or mesothelial. It is not highly probable that mesenchymal cells have wandered there from the embryonic body (after the heart had formed), much less likely from the distantly located intermediate cell-mass. Whatever may have been their ultimate source, certain it is that blood-cells may be found in the hearts of embryos which have never had a blood-circulation, although Stockard has made statements to the contrary. An unusually interesting case is that of an embryo kindly placed at my disposal by Dr. E. I. Werber. Here, instead of one haemophoric dilation or even two as I had sometimes found, there are several, all separated by constricted solid regions. This embryo had never developed a circulation.

I have postponed so far an account of the rather interesting history of the heart of the embryo from which figure 1 is taken. On the fifth day it was observed that the heart of this embryo

possessed solid extremities. Prior to that time it was definitely known that there had been no blood-circulation. At this time it was decided that a procedure might be resorted to by means of which one might be assured that no circulation would take place. The very feebly pulsating heart possessed a flexure and was not at all stretched, yet the solidity of its venous end could not be doubted. A very fine needle was introduced into the pericardium and the mid-portion of the heart was carefully severed. The cut ends then lashed aimlessly about. At the next observation it was found that by some happy chance the free ends had overlapped and fused laterally and heart-pulsation had ceased. When the embryo was sectioned the fusion was found to be quite intimate in places, in others rather loose, as in figure 23. The rounded portion in the left of figure 23, if traced anteriorly, is found to be the venous portion of the heart. It becomes solid, and contains here and there a few erythrocytes, singly or in small groups. The small rounded portion of the heart on the right side of figure 23, if traced anteriorly for about thirty micra, disappears. If it be traced posteriorly it becomes larger and solid with intermittent groups of erythrocytes. In the 'venous portion' (left side of figure 23), the outline of the endocardium is evident. Its cells have, however, become cuboidal, and even columnar. The cavity on the right side of the figure contains, as in figure 19, well differentiated erythrocytes; some of them are still in a process of differentiation and somewhat basophilic.

Encouraged by the success of this experiment, I performed about fifteen hundred cardiectomies on normal Fundulus embryos, in all cases removing the heart anlage previous to its pulsation or even before a well defined heart could be made out. The percentage of fatality was high indeed, but a few embryos survived to the age of nine to twelve days. The exceedingly gratifying results of these experiments are reserved for a later publication.

So far I have offered no suggestion as to the source of erythrocytes in hearts containing erythrocytes which were not carried there passively by body-fluids in motion. The conditions to be observed in figures 24, 25, and 26 give a clue as to the possible

source of the erythrocytes which originate locally in the heart. The sections are from the embryo of which figure 7 represents the dorsal cephalic surface on the eleventh day of the embryo's history. Not realizing the extraordinary future of this embryo, I did nothing further than to keep it segregated with certain other embryos which had received like chemical treatment and from which all embryos acquiring circulation were removed. I feel sure that no circulation was ever developed in this embryo. As soon as distinct endocardium could be made out, this embryo with several others was further segregated to a dish into which only those embryos were placed whose otherwise normal endocardia were solid at both extremities. On the eleventh day the upper portion of the heart had acquired a faint orange color which gradually deepened until the embryo was killed on the fourteenth day. Not only was the existence of blood-producing areas as seen in figure 7 satisfactorily evinced, but the conditions in the heart were of unusual interest.

Figure 24 represents a section through the heart of this embryo in which a distinct and complete endocardium can still be made out. The endocardial cells have become decidedly cuboidal in outline, as in figure 23; the contained erythrocytes are in an eosinophilous column continuous with those eosinophilous cells of figures 25 and 26.

In the right of figure 25 it will be noted that an active haematopoesis has taken place, and that both endocardium and myocardium have been involved, whereas the mesothelium surrounding the myocardium has remained unaltered. In figure 26 it will be seen that both the endocardium and myocardium have in this region become completely transformed into strongly eosinophilous erythroblasts, the crowding of which has prevented the rounding of their contours.

From the foregoing considerations it is evident that red blood-cells can develop in the anterior mesenchyme or in the anterior vessels under conditions in which heart pulsations could not have accounted for their observed position, whether the heart had acted as a force-pump or as a vacuum-pump. Especially is this

true of the embryo from which figures 16, 17 and 18 were made. It is evident also that cardiac tissue can produce blood-cells.

If these foregoing observations are correct, their significance is so apparent as to call for little discussion; with the premises upon which Stockard bases his support of the polyphyletic theory these observations seem incompatible.

I am willing to admit that a very large percentage of the embryos, under certain sorts of chemical treatment,[5] may yield conditions which would conform to Stockard's conclusions. But I find also that in order to suppress conclusions diametrically opposed to those of Stockard, I must fail to take account of the exceptions to his sweeping generalizations. The situation is quite simple; exceptions can undoubtedly be found. I cannot regard such exceptions as mistakes of nature and devoid of significance.

I realize, as I did in my previous experimental work on chick blastoderms, that "it is not always possible to determine the extent to which experimental conditions portray a truly normal process," but I do claim for the experimental method that it possesses true value in breaking down certain dogmatic and preconceived negations which we have been prone to attach to the potentialities of the mesodermal derivatives.

Once we have seriously canvassed the potentialities of a tissue we are then in position to determine, in an unbiased manner, the extent to which the prospective significance of that tissue normally coincides with its potentiality.

ADDENDUM

Since the preparation of the foregoing account, I have been so fortunate as to receive Dr. Stockard's latest publication in the American Journal of Anatomy, vol. 18, no. 2. So elaborate in

[5] In running a series of many thousand embryos for Professor McClure I have never found a case in which an alcoholic embryo displayed this anterior haematopoesis in the living condition. It will be noted that practically all my embryos were treated with butyric acid or acetone; they were reared in running sea-water. Stockard has told me that his embryos were reared in a very small amount of sea-water in an environment much warmer than that of my experiments. The hatching-period of my embryos was relatively about two weeks later than those of Stockard's experiments, so that our time-relations, at least, are not comparable.

its nature and so minute in its detail, this article seems at first to render rather futile my own work.

"In reply to the extreme monophyletic position," Stockard (p. 315) asks the following questions: 1) Why are only erythrocytes present in old blood-islands on the yolk of non-circulating specimens? 2) Why is no cellular blood element present in the aorta and other endothelial lined vessels in the anterior region of similar embryos? 3) Why are the wandering primitive blood-cells unable to form blood in the liver and other positions, while blood-forming power is present to a certain extent in certain regions of the same embryo but from a different anlage?

In reply to this equally extreme polyphyletic position I give the following answers: 1) It is possible to find in the yolk of certain chemically treated embryos disintegrating or at least abnormal cells which bear a striking resemblance to certain cells in the anterior end of the embryonic body which Stockard would probably call white blood cells, but which I consider as abortive attempts at erythrocyte formation and which have, or have had the potentiality of taking on a haemoglobin content under favorable conditions. 2) Embryos entirely devoid of complete circulation may have erythrocytes within or outside their endothelium in the anterior end of the body. Why such blood cells often fail to enter or become surrounded by endothelium or are generally much less typical, and display such a very transient period of redness as compared with the erythrocytes of the intermediate cell-mass, is difficult to explain. It may be merely an expression of the extreme general susceptibility of the anterior end of the embryo to the action of toxic substances—a susceptibility which Child has ably considered under the term 'axial gradient.'[6] Werber saw the application of Child's useful work to the explanation of cyclopia. It is evident that the erythrocyte anlagen share in this sensitivity. From this consideration, the failure of the anterior blood-cells to come to full realization of their potentialities should be an occasion for no greater surprise than the circumstance that the anterior nervous, alimentary, endothe-

[6] Child, C. M., Jour. Exp. Zool., vol. 13.

lial, or skeletal tissue may be very completely upset while in the posterior region it is perfectly normal (see many of Stockard's figures). In about 100 per cent of the survivors of cardiectomies which I have performed, on normal embryos where the anterior region suffered no specific injury the erythrocytes in this region, so far as I can detect, are in no way morphologically different from those of the posterior region of the embryo, or those of the yolk-sac including those on the anterior portion of the yolk-sac. 3) In reply to this question I wish to place the following counter-interrogation: Why does Stockard admit that "the only cells within the embryo which resemble lymphocytes and leucocytes in their general structure and staining capacities have been found in the anterior portions of the body and in the head region of young embryos" (p. 280)? What assurance beyond mere inference does Stockard give that these cells (of his figures 45–48) 'resembling leucocytes' are of a roving disposition and "finally (p. 284) become scattered throughout the embryo's body?" One must admit that he has effected a skillful transition from his limited facts on this point to his general conclusions. Concerning the haematopoetic potentiality of the liver, I shall later have some facts of interest to communicate. I regard the term 'haematopoetic organ' a misnomer as applied to early embryonic development.

Stockard's difficulty may have arisen from a misinterpretation of the potential, abortive, or abnormal erythroblasts in the anterior end of the embryo. The word erythrocyte means red cell, while the word leucocyte means white (or colorless) cell. When I have been able to locate these cells by their red color in the anterior end of the living embryo which has never had a circulation, and can predict their position in section (a feat to which I am unequal in case of leucocytes) I feel fairly sure of my ground. Such cells correspond exactly in position to Stockard's leucocytes, lymphocytes, and leucocyte-like cells, and may resemble them in abnormal cases in their staining reaction.

I am not sure that any of Stockard's white blood cells are really such. I have never found a cell in these abnormal embryos which I have felt sure could be considered to be a white blood cell. From

the point of view of the monophyleticist it would be very desirable for these erythroblast anlagen to transform into leucoblasts.

It is of interest to note Stockard's dual attitude towards the time of appearance of white blood cells. On page 265 their very early appearance fits in nicely with his theoretical considerations, while on page 309 their late appearance in ontogeny is fraught with significance.

I regard as a vulnerable point in Stockard's line of reasoning the fact that he in no way proves that the erythrocytes of the yolk-sac are always descendants of the material of the future intermediate cell-mass but in a vague manner derives them from mesoderm "related to the intermediate cell-mass." What assurance does he give us of the specificity of this mesoderm?

When Stockard finds a blood-cell not comforming to his ideals (p. 395) he regards its position as 'exotic.' Given such license one can prove anything. Such reasoning from authority could even be rendered disastrous to our experimental 'proof' of the local origin of intraembryonic endothelium.

Stockard argues with perfect justice that the demonstration of diverse types of differentiating blood cells in close physical proximity does not necessarily indicate that the cell which gave rise to one sort of blood-cell could have ever given rise to a different kind of cell. One must admit, however, that the benefit of the doubt here falls to the monophyletic view. It might be argued with equal fervor that the precocious formation of cells of a given type in a definite region does not necessarily prove that the fate of those cells had been sealed ab initio. One must admit, however, that the benefit of the doubt under these circumstances falls to the polyphyletic view (neglecting for the moment the fact that the segregation is by no means so precocious as Stockard's account would indicate). Any such segregated cells might have given rise to a different sort of cell if placed in a different environment at a sufficiently early period of development.

I have previously plead that the development of the blood-vascular system "furnishes an unproductive field for the solution of the problems of preformation and epigenesis." The

point which I hope to help prove is that practically all regions are capable of furnishing their own vascular tissues, endothelial and corpuscular. Those regions which do so to an extreme degree are all important but not all-important sources of the vascular tissue. The process is essentially one of transformation of morphologically indifferent mesenchyme cells in loco. That there is a specific cause underlying each transformation follows as a matter of course. I am not at present concerned with the possibility of those mesenchyme cells having migrated from some other position; the present situation is relieved of such perplexing considerations when Stockard goes so far as to say that the heart, anterior vessels, anterior yolk, and liver are regions in which wandering blood anlagen never make themselves manifest; he also speaks of erythrocytes 'originating' on the yolk when it is certain that their ultimate anlagen did not occupy that position. Neither would an authoritative extension of the intermediate cell-mass to include the region of the eyes and snout affect the main question at issue—the question whether the anterior region and certain other regions are necessarily devoid of blood-cells if the embryo has had no circulation.

It is not impossible that the embryo represented in Stockard's figure 9 possessed haemophoric precardinals. A further study of this embryo might point to the possibility that the haemophoric vessel which he has labeled as cardinal vein is really the duct of Cuvier with its anterior continuation, the precardinal. In his figure 13 the haemophoric 'cardinal vein' runs as far anteriorly as the otocyst. One should certainly not expect to find the cardino-Cuverian junction farther cephalad than the posterior limits of the otocysts. It is possible also that erythroblasts not possessed of sufficient haemoglobin to render them visible in the living embryo might be found in sections of these specimens still farther anteriorly. If these inferences be correct it is obvious that Stockard's own material does not bear out his conclusions. Stockard does not give in detail the individual treatment of each embryo but from his account of a typical experiment (p. 234) it would seem that the embryos were, in some cases, left in a fairly strong alcoholic solution until twenty-four hours prior

to the time at which they were preserved for study. It does not seem probable that such an environment would be favorable to the elaboration of the complex haemoglobin molecule, which, even when once formed, is very susceptible to the action of alcohol. It is possible that some regions might, under favorable conditions of oxidation, be able to produce erythrocytes even under these circumstances while other regions might not.

It is interesting to note that in my figure 8 (a condition rarely found) the posterior region seems to have been the part affected by toxic action; this part of the body is diminutive in size whereas the anterior end is relatively normal. Correlated with these conditions we have a complete absence of erythrocytes in the tail-region of this embryo and an abundance of them in discontinuous lines into the ophthalmic region.

Once the main fact of the local origin of vascular tissues has been established, it is perfectly agreeable to me that we speak of 'diffuse anlagen' if the incontrovertible facts are more acceptable under that phraseology. It would be desirable indeed to permit of all conceivable grades and degrees of 'diffuseness' to suit all occasions.

Still more recently I have received Stockard's communication in the *American Journal of Anatomy,* vol. 18, no. 3. In the main I can confirm his observations on the living yolk-sac and have watched the same process in the tail of the living embryo. My metaphysical deductions differ to a certain extent from those of Stockard as may be seen by comparing his preformistic conclusions with a discussion which I have submitted for publication in the proceedings of the American Association of Anatomists for the current year.

I wish to call attention to the seemingly exotic vitelline vein of Stockard's figures 23 and 24 in his latest publication. Here is shown a vitelline vein undoubtedly comparable to that of his figure 26 which arises in the cephalic region. I strongly suspect that this vitelline vein is a duct of Cuvier. In this connection see p. 116 of my present publication, and Reagan and Thorington, figure 15, in the same number of the Anatomical Record. To anyone confused on this point I recommend a careful study

of Wenckebach's excellent diagrams in Hertwig's Handbuch, Erster Band, Erster Teil, zweite Hälfte, p. 1131.

I note with especial satisfaction the contents of Stockard's page 586, *American Journal of Anatomy*, vol. 18, no. 3, where he states that a loss of circulation has little or no tendency to cause a vessel to collapse or to become obliterated. I have removed the hearts of embryos which possessed a circulation; later I have been greatly surprised to find that the main endothelial tubes had persisted in a remarkable manner as long as the tissue of such embryos remained alive. It is not a rare thing to find embryos which, in addition to having blood in their anterior ends, display the following conditions: ventral and dorsal aorta and aortic arches absent; heart solid; anterior end of the body and anterior yolk devoid of endothelium. From these considerations it seems that Stockard's precautions on pp. 578–9 (ibid) are superfluous if the tissue be well preserved and sectioned.

A major claim of Stockard's latest work is that all the wandering cells on the yolk-sac have the same environment. I regard such a claim as thoroughly unscientific even when it is applied to any two cells in any stage of ontogeny.

PLATES

PLATE 1

EXPLANATION OF FIGURES

1–9 are dorsal views of the cephalic portions of chemically treated embryos. Figures 9 and 10 are lateral views. The stippled areas in these figures indicate the location of erythrocytes as observed in the living embryo.

1, 2, 5, and 7 represent the heads of fourteen-day embryos, which at their four-cell stage were treated for twenty-four hours with a solution of 50 cc. sea-water to which had been added 15 cc. of M/12 butyric acid. They are all from the same experiment, one-fourth of the embryos in which yielded similar results. Owing to the evanescence of the red coloring, it is possible that many others may have passed through this critical stage without being observed.

3 and 4 are described with sufficient detail on page 103.

8 and 9 are from embryos obtained from the same experiment as that of figures 3 and 4.

10 is from an embryo, the treatment of which is described on page 104.

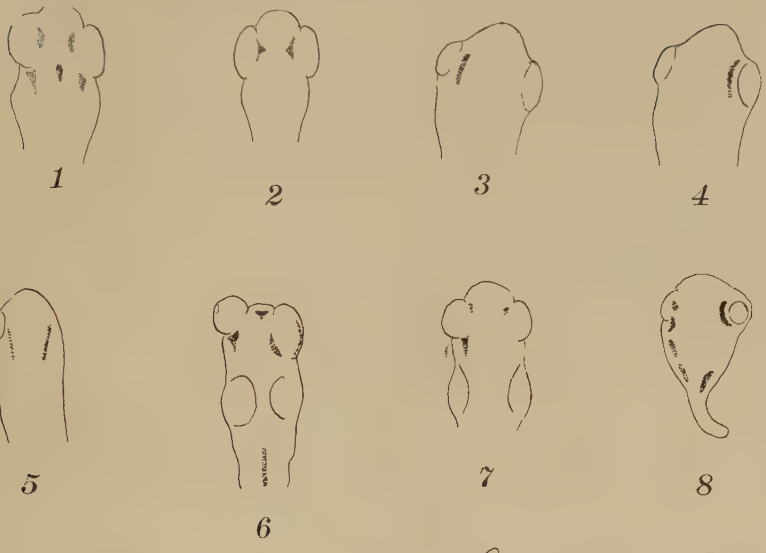
11 represents an embryo, only a small portion of the body axis of which developed. No heart formation has taken place. There is a large dilated pericardium in the region of which are many isolated erythrocytes. Embryo from same experiment as that from which the embryo of figures 1, 2, 5, and 7 were obtained. It seems improbable that the 'pericardium' of this embryo should be interpreted as Kupffer's vesicle. Even so, there are blood cells of local origin on what would then be the anterior yolk, not shown in this figure.

12 represents a case in which the embryonic body has failed to develop, or as such has been transformed into mesodermal and vascular tissues which are scattered entirely over the yolk. The embryo at the four-cell stage was treated for ninety-six hours with a solution of 50 cc. of sea-water to which was added 15 cc. of a molecular solution of acetone.

ABBREVIATIONS

Erth., Erythrocytes

Ht., Heart


1
2
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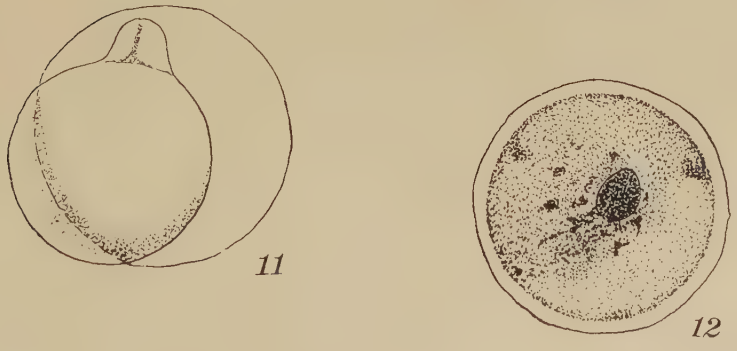
9
Erth.
Ht.
10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12

PLATE 2

EXPLANATION OF FIGURES

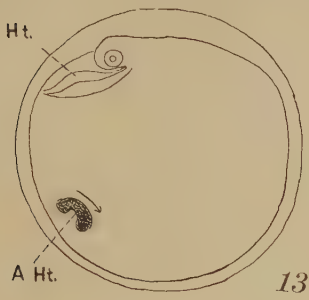
13 and 14 are described in detail on page 105. The small arrows indicate the direction of pulsation in the 'accessory heart.' Note the apparently normal condition of the actual heart.

15 is a lateral view of a twelve-day embryo treated for twenty-four hours subsequent to the four-cell stage with a solution similar to that employed in case of the embryos represented by figures 1, 2, 5, 7 and 11—kindness of Dr. E. I. Werber.

16 is a transverse section through the optic region of a nine-day embryo which at the four-cell stage was treated with a solution of 50 cc. sea-water, to which had been added 15 cc. M/12 butyric acid. The yolk-sac is devoid of vascular tissue. The space in the optic cup (on the left side of the figure) normally occupied by a lens, contains a blood lacuna. On the right side is seen the attachment of the upper optic stalk and the base of the lower.

ABBREVIATIONS

A.Ht., 'Accessory' heart
Bl.l., Blood lacuna
End.C., Endocardium
Erth., Erythrocytes
Ht., Heart
L., Lens
My.C., Myocardium
O.C., Optic cup
O.st., Optic stalk



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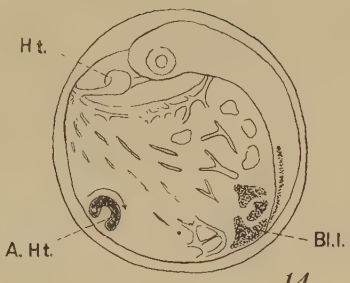

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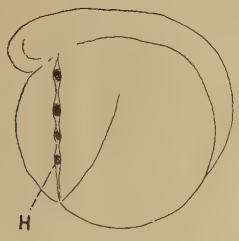
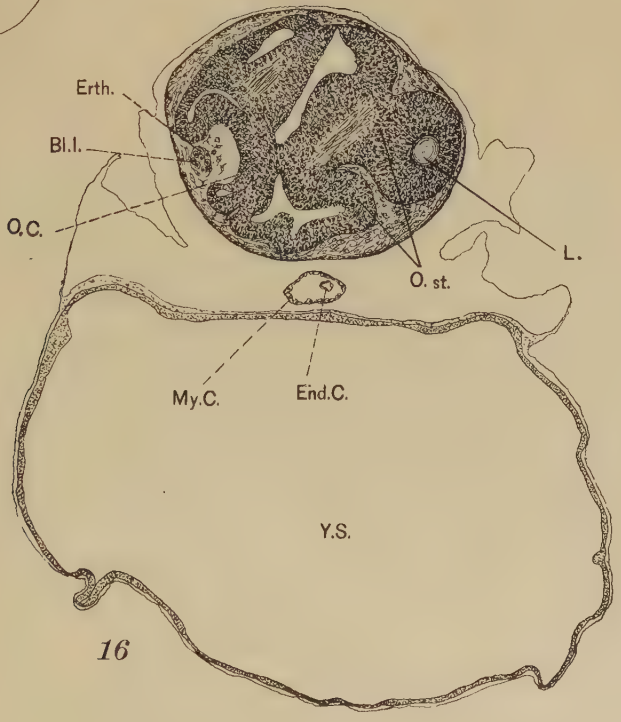


15



16

PLATE 3

EXPLANATION OF FIGURES

17 is a transverse section of the same embryo from which figure 16 is taken; it is a section passing through the optic anlagen, showing isolated erytrocytes, solid heart, and unusual eye-formation.

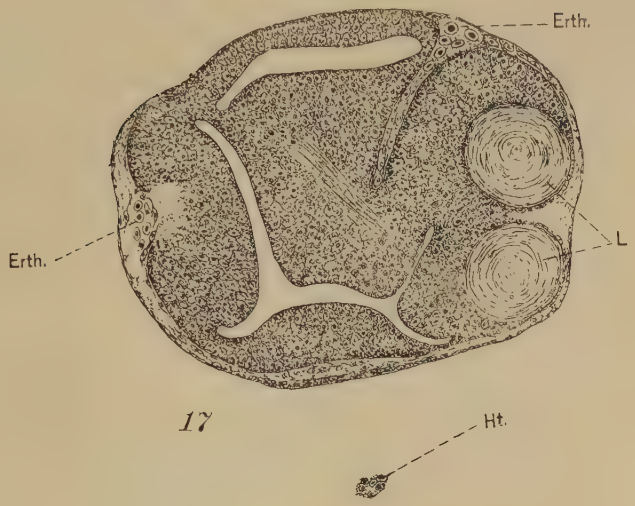
18 is a section of the same embryo slightly posterior to the optic stalks, showing the pre-cardinals as haemophoric vessels. The erythrocytes are angular in contour (especially in the vessel on the right of this figure) due to their crowded condition A few of the erythrocytes in the less crowded vessel on the left have become rounded.

ABBREVIATIONS

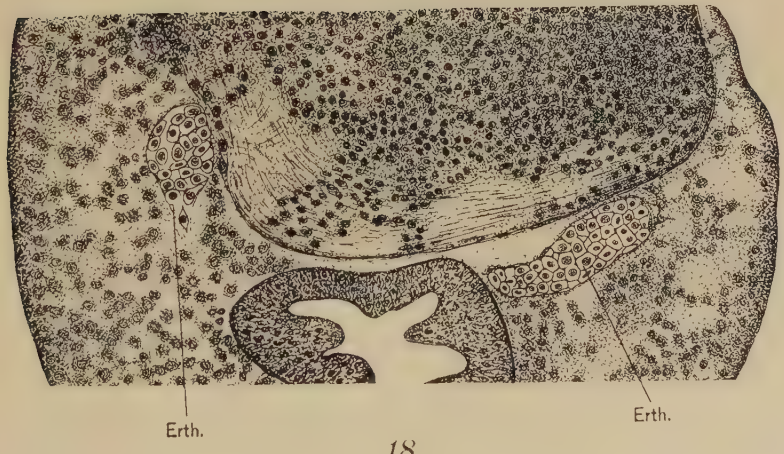
Erth., Erythrocytes

Ht., Heart

L., Lens



17



18

PLATE 4

EXPLANATION OF FIGURES

19, 20, 21, 22 and 23 are transverse sections of the embryo from which figure 1 was drawn. Figure 19 is a section just posterior to the optic cups passing through the anterior ends of the haematopoietic areas of this region as shown in figure 1. In this figure, as in figure 20 the position of the blood-cells is roughly and arbitrarily indicated by heavy black dots.

20 is a section passing through the posterior portions of the optic cups, and shows the position of the median blood anlage of figure 1. The heart in this figure is a solid continuation of the left portion of the double body labeled heart in figure 19.

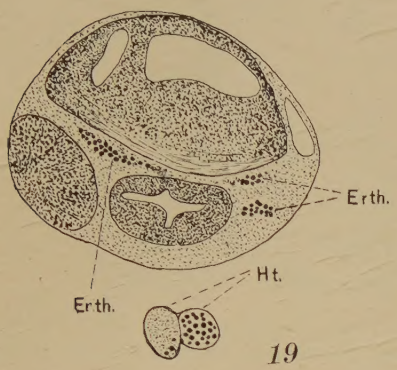
21 is an accurate detail of the blood-producing areas seen on the right side of the head in figure 19. Conditions in the lower lacuna seem to be farther advanced. Certain mesenchyme cells (lightly stippled) are in a stage of transition to erythrocytes (cytoplasm clear). A blood space is forming while the mesenchyme cells bounding it seem to be receding to form endothelium. The free erythrocytes are rounded in contour.

22 is an accurate detail of the median dorsal blood anlage of figure 20. The blood-cells are very strongly eosinophilous but are crowded and possess angular contours.

ABBREVIATIONS

Erth., Erythrocytes

Ht., Heart



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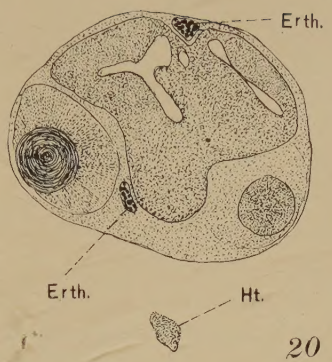

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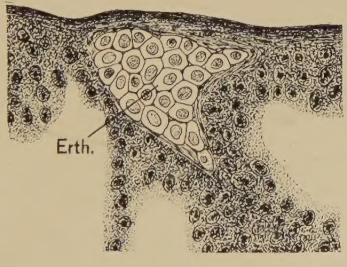


22

PLATE 5

EXPLANATION OF FIGURES

23 is a section through the heart of this same embryo near the plane of section of figure 19. The connection of the fused portions as described in the text, is looser than in the plane of figure 19. The right 'arterial' side contains no endothelium in this section. Erythroytes in various phases of development are observed. Note the cuboidal nature of the endocardium in the left side of the figure.

24 is a section through the heart of the embryo from which figure 7 is made. In this figure the endocardial cells are cuboidal and rather deeply staining. The endocardial cavity contains erythrocytes which are in a column continuous with the erythrocytes of figures 25 and 26. There is a more or less distinct myocardium.

25 shows a section of the same heart in which a portion of the endocardium and myocardium are transforming into eosinophilous blood-cells.

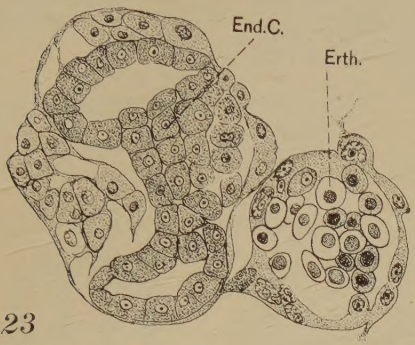
26 is from the same heart The plane of section is near the upper end of the heart. The entire endocardium and myocardium of this region has transformed into eosinophilous blood-cells which, as in many instances already noted, are unable to round themselves out for lack of space.

ABBREVIATIONS

End.C., Endocardium

Erth., Erythrocytes

My.C., Myocardium



23

24

25

26

BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

ANATOMY OF THE BRAIN AND SPINAL CORD WITH SPECIAL REFERENCE TO MECHANISM AND FUNCTION. For students and practitioners. Harris E. Santee, A.M., M.D., Ph.D., Professor of Nervous Anatomy in Chicago College of Medicine and Surgery, Medical Department of Valparaiso University; Professor of Anatomy in Jenner Medical College, Chicago. Fifth edition, revised and enlarged with 158 illustrations, 46 of which are printed in colors. $4.00. Philadelphia: P. Blakiston's Son & Co., 1012 Walnut Street.

GRUNDZÜGE DER VERGLEICHENDEN GEWEBELEHRE. Dr. Med. Friedrich Maurer, O. Professor der Anatomie und Direktor der Anatomischen Anstalt in Jena. 486 pages including Index, 232 figures. Leipzig: Verlag von Emmanuel Reinicke. 1915.